PROCEEDINGS

21ST SOUTHERN FOREST TREE IMPROVEMENT CONFERENCE

June 17-20, 1991

KNOXVILLE , TENNESSEE
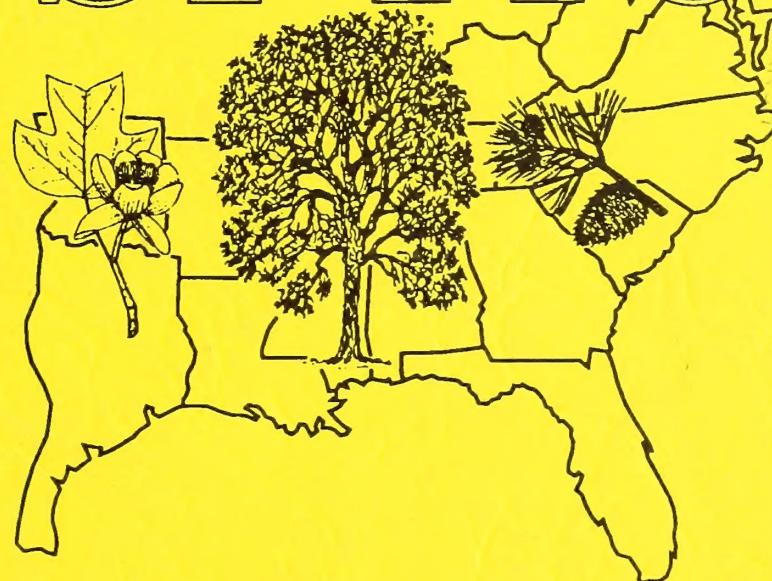
SPONSORED BY
THE SOUTHERN FOREST TREE IMPROVEMENT COMMITTEE
IN COOPERATION WITH
THE UNIVERSITY OF TENNESSEE
AND
TENNESSEE DIVISION OF FORESTRY

PROCEEDINGS OF THE

TWENTY-FIRST SOUTHERN FOREST TREE IMPROVEMENT CONFERENCE

JUNE 17-20, 1991

Knoxville, Tennessee

Sponsored Publication No. 43 of the

Southern Forest Tree Improvement Committee

PREFACE

The 21st Southern Forest Tree Improvement Conference was held at the Hyatt Regency Hotel in Knoxville, Tennessee. The conference was sponsored by the Southern Forest Tree Improvement Committee, in cooperation with the University of Tennessee and the Tennessee Division of Forestry. There were 161 attendees representing 26 states and four foreign countries.

A total of 42 presentations, nine invited and 31 voluntary, were given. The voluntary papers were evaluated by the Southern Forest Tree Improvement Committee and three referees to receive the $200 Tony Squillace Award for the best oral presentation/written paper. The paper "Tree Size Influences Flowering Response in Juvenile Loblolly Pine" by Leon Burris, Claire Williams and Susan Douglass, was selected to receive the Squillace Award. Congratulations to these authors for an outstanding contribution.

Thirteen posters were exhibited during the poster session. The Baruch Foundation of Georgetown, South Carolina again sponsored a $100 dollar award for the best poster. Roger J. Arnold was selected as the recipient of this Baruch Forest Science Institute Award for his poster "Genetic Improvement of Pinus radiata Combined with Integrated Nursery and Outplanting Systems Lower Plantation Establishment Costs."

Two night sessions were held that involving group discussions on selected topics. The Seed Orchard Managers Meeting was chaired by Tom Tibbs. Ron Dinus organized the discussion on the inclusion of Forest Biotechnology subject matter into undergraduate and graduate curriculums.

21st SFTIC Conference Committee:

R. A. Cox - Tennessee Division of Forestry
R. J. Dinus - Institute of Paper Science & Technology
J. L. McConnell - U.S. Forest Service
R. Rousseau - Westvaco Corporation
S. E. Schlarbaum - University of Tennessee
G. A. Tuskan - Oak Ridge National Laboratory
D. B. Wagner - University of Kentucky
R. J. Weir - North Carolina State University
T. L. White - University of Florida

The papers in these Proceedings have been published as submitted by the authors, in camera-ready form. Responsibility for the technical content remains with the respective authors.

Copies of this publication can be obtained from:

The National Technical Information Service
Springfield, VA 22161

CONTENTS

GENERAL SESSION II: Seed Orchard Management

GENERAL SESSION III: Breeding and Propagation

WELCOMING
ADDRESSES

STATE FORESTER'S WELCOME

21st SOUTHERN FOREST TREE IMPROVEMENT CONFERENCE

Roy C. Ashley
Tennessee Department of Agriculture
Division of Forestry

- FIRST let me extend a warm and hearty welcome to the participants of the 21st Southern Forest Tree Improvement Conference!
- We in Tennessee are happy to host you and hope that your visit with us will be productive.
- I know Russ Cox and Scott Schlarbaum will do all they can to make you feel at home.
- I was recently reminded by Russ that almost one year ago he had asked me to extend this welcome to you today.
- Now --obviously I'm not a geneticist, so I won't pretend to be an expert in the field.
- However, what I can do is offer you a challenge (or encouragement) for the future.
- In thinking about what I should say, I took the liberty of reflecting on Tennessee Division of Forestry's past.
- 29 years ago I started to work for this agency.
- At that time there was no official Tree Improvement program.
- Since that time we have made progress.
- Today we have a program.
- AND -- we are aware of the benefit that a good Tree Improvement program can bring to the forest landowners.
- We have made this progress
        *Without a large Tree Improvement staff
        *Without a large Tree Improvement budget
- What we have had is a few individuals dedicated to their cause.
- As a result, our agency now has:
        *250 acres of seed orchards
        *300 acres of forest genetics studies
        *AND---much of our seedling production comes from our own
         orchards.
- In the mid 1980's we decided to take a look at our Tree Improvement Program.
- Because we were able to recognize our limited resources early, two important things resulted:
        1) We developed a Memorandum of Understanding with the University of Tennessee that more clearly defined the two agencies roles in Tree Improvement and allows us to combine our resources into one program--Tennessee's Tree Improvement Program.

1

2) A document was developed with the help of various Tree Improvement "experts" inside and outside the state, that helped us <u>focus</u> where Tennessee needs to go in the future in forest genetics.

- The goals of Tennessee State Government today are three-fold, to improve:
  - Economy
  - Environment
  - Education----the three E's!
- I think Tennessee's Tree Improvement Program fits those goals well.
- Although you will understand, most people would not, when I say that an effective Tree Improvement Program is an important part of maintaining that <u>economic</u> focus.
- More people would understand that trees are important to the <u>environment</u>. Therefore, I'm pleased to see your keynote session entitled <u>"Tree Improvement and Environmental Change"</u>.
- <u>AND</u>--what about <u>Education</u>, that third "E"? We <u>all</u> have a tremendous challenge to educate the public on not only the <u>value</u> of trees, but also to the importance of <u>improved</u> tree species.
- <u>We know we can use trees to</u>:
  - *Improve regional economics
  - *Improve the quality of life of our citizens
- What <u>public</u> doesn't know is that Tree Improvement is an important, but <u>almost unseen</u>, part of the puzzle in maintaining economic growth and overall quality of life through the use of trees.
- <u>However</u>, with the tree improvement specialist, as with other specialists, dedication to a cause told you to press on--for the good of the resource.
- Why, in my opinion, should the Southern Forest Tree Improvement Conference exist? <u>Four reasons</u>:
  1) To set Direction
  2) Inform/Educate
  3) To Protect and Enhance our Future Forests
  4) To Encourage Good Forest Management
- In setting the <u>direction</u>, you, the expert, must help chart the course in creating our future forest.
- In <u>informing/educating</u>, you, the experts, can help make the public aware of the value of improved trees, both economically <u>and</u> environmentally.
- You, the expert, can help <u>protect</u> our future forests by the improved trees you develop.
- You, as experts, can encourage proper forest management by creating better trees than those that currently exist.
- <u>So</u>--in short, your improved trees can become an incentive to landowners to properly reforest their lands!
- And in doing so, you can improve the <u>visibility</u>, <u>viability</u> and <u>versatility</u> of a very important tree program that benefits all society.
- The reality is this! In today's economy none of us can go it alone.
- There's not enough <u>people</u> or <u>money</u>.

- So--we need the coordinated approach that this conference can provide.
- Some of you can provide basic research.
- Others, applied research.
- We need public and private Tree Improvement Programs that join hands to accomplish mutual goals.
- I assume that's partly why this diverse group is here today.
- A sharing of information can move us ahead faster, cheaper, and with a better overall focus---for the betterment of our regional forest resource.
- How do you meet the challenge? Two Suggestions:
    1) "STRETCH" Message
        * Consider a rubber band.
        * It can stretch to encompass many different things.
        * Our Tree Improvement Program must do the same.
        * For example:
            We are good at providing improved pine species
        * BUT--there is a new challenge before some of us.
        * The challenge of providing quality hardwoods for reforestation.
        * AND--then there is the challenges of global warming, greenhouse effect, urban forestry and fiber production, as they relate to healthy and viable forests.

    2) QUALITY MESSAGE
        * Quality is the building block in the foundation on which all Tree Improvement Programs stand, and
        * The quality of our seedlings is the attribute by which we will be measured.
        * Without it, we provide a substandard service.
        * With it, we provide an exceptional service to our public.
        * SO--let's continue to strive for quality in our Tree Improvement Programs as we move ahead in this meeting and back home in the workplace.

- I hope your stay in Tennessee is informative and pleasant.
- I know Russ and Scott have worked hard to provide a good program.
- THANKS for the opportunity to visit.
- AND ---
- Come back to see us soon to work or play.
- REMEMBER--Tennessee is a good place just to visit!

Welcome from the University of Tennessee

Eyvind Thor
Professor Emeritus
Department of Forestry, Wildlife & Forestry
The University of Tennessee, Knoxville

Welcome. I hope that by now you all realize that the people in Tennessee want you to feel welcome here. Nearly half an hour has been put aside for this purpose. Still, if any of you should feel under-welcomed when I am finished; please feel free to talk to me when we break for lunch.

As some of you know, for several years I was active in the sponsoring organization of this conference - The Southern Forest Tree Improvement Committee. Also, I and a number of my graduate students regularly presented papers at conferences. Of the many scientific meetings which I attended during my career, the conferences of the Southern Forest Tree Improvement Committee always were the most important and enjoyable. It was not only the learning of research in progress, but also the excitement of renewing friendships, delight in the latest gossip, and somehow feel that you were a member of a very special fraternity.

My introduction to The Southern Forest Tree Improvement Conference came 30 years ago when I gave a paper based on my dissertation at N.C. State Univ. In retrospect, it was a disaster. This was my maiden voyage and I sank, with my cargo of crummy slides nobody could read even if they were seated 10 feet away from the screen. But a most wonderful thing happened when I staggered down from the podium, everybody clapped vigorously. During coffee break several members came over, introduced themselves and professed that it was a most interesting paper. God bless those liars, they sure made a budding scientist feel good!

Since that time, the conferences became very dear to me. It was a forum where graduate students could present their work and expect kindness. It was also a place where mature scientists could expect to be questioned, sometimes without much kindness. Today's conference is the twenty-first. Since you meet every other year, the first conference was held 40 years ago. Perhaps as many as half of you were not even born then. If you have any doubt about the progress made in those 40 years, you should pull out copies of those first Proceedings. The most exciting thing you will find is the result from some seed source test. Looking through the Program for the 21st conference, it is clear that you have gone a long way from those good old days when life was more simple.

However, one thing has not changed. Whatever plant material we produce, be it by conventional breeding methods or genetic engineering, it must be placed in an environment where it can grow and perhaps, make a profit. Silviculturally speaking, this means clearcutting the existing forest and planting seedlings. Forty years ago, there was no criticism of this method. As a matter of fact, it was a sign of advanced forestry. Today, so called environmentalists are putting political pressure on forest resource managers to use methods resulting in uneven-aged forests.

The U.S. Forest Service is most exposed to such pressure, because it manages large areas of public lands. From what I read in the newspapers, the Forest Service has pretty much caved in to the "environmentalist" demands. I am afraid our forestry schools are next. There is no better way to stop clearcutting than getting the forestry graduates indoctrinated in this "New Perspectives" forestry.

Considering this, I will leave you with this question: Do you think that 40 years from now there will be a 42nd Forest Tree Improvement Conference?

KEYNOTE SESSION

TREE IMPROVEMENT
AND ENVIRONMENTAL CHANGE

GLOBAL CLIMATE CHANGE: SOME IMPLICATIONS, OPPORTUNITIES, AND CHALLENGES FOR U.S. FORESTRY

G. Marland[1]

Abstract.--It is widely agreed that the concentration of greenhouse gases in the earth's atmosphere is increasing, that this increase is a consequence of man's activities, and that there is significant risk that this will lead to changes in the earth's climate. The question is now being discussed what, if anything, we should be doing to minimize and/or adapt to changes in climate. Virtually every statement on this matter; from the U.S. Office of Technology Assessment, to the National Academy of Science, to the Nairobi Declaration on Climatic Change, includes some recommendation for planting and protecting forests. In fact, forestry is intimately involved in the climate change debate for several reasons: changing climate patterns will affect existing forests, tropical deforestation is one of the major sources of greenhouse gases to the atmosphere, reforestation projects could remove additional carbon dioxide from the atmosphere, and there is renewed interest in wood-based or other renewable fuels to replace fossil fuels. Part of the enthusiasm for forestry-related strategies in a greenhouse context is the perception that forests not only provide greenhouse benefits but also serve other desirable social objectives. This discussion will explore the current range of thinking in this area and try to stimulate additional thinking on the rationality of the forestry-based approaches and the challenges posed for U.S. forestry.

Keywords: Climate Change, Carbon Dioxide, Reforestation

On June 23, 1988, with the U.S. in the midst of a major East Coast drought, Jim Hansen of the National Aeronautics and Space Agency went before a Senate Committee and stated that he was 99% sure that the global climate was changing and that the change could be attributed to the increasing concentration of greenhouse gases in the atmosphere (Hansen 1988). Since that day global climate change has been an increasingly important political issue in the U.S. and around the world. Within 7 months we saw bills in the U.S. Congress like Senate Bill 201, which asserted "The Congress finds that the Earth is a fragile planet with a thin blanket of air, a thinner film of water, and the thinnest veneer of soil to support a web of life", and proposed a host of remedies to slow global climate change. Carl Sagan has referred to this particular assertion as "one of the most important findings of the Congress in 200 years" (Sagan 1989) but the political will is not yet firmed to pass such sweeping legislation in the face of continuing uncertainty about the magnitude and impact of global climate change. Nonetheless, there has been continuing debate, both domestic and international, on what, if any, actions should be pursued, and forestry is a central component of most action proposals. I would like to take a minute to dramatize the political perception of forestry's role and then back up and look at the essence of the climate change issue and the challenges and opportunities it offers for U.S. forestry.

This is a sampling of the smorgasbord of observations and declarations now in the international literature. From the America the Beautiful Plan to plant trees in the U.S.

(U.S. Department of Agriculture 1990), "President Bush is proposing a major new initiative, called America the Beautiful, that provides an environmental legacy, enhances existing natural and recreational resources, and addresses mounting public concern about the buildup of atmospheric carbon dioxide". From the Noordwijk Declaration, a 67-country ministerial declaration (1989), "...agrees to pursue a global balance between deforestation on the one hand and sound forest management and afforestation on the other. A world net forest growth of 12 million hectares a year in the beginning of the next century should be considered as a provisional aim." From the Nairobi Declaration on Climatic Change (1990), "Governments of African countries are called upon to adopt and implement policies which...initiate and promote afforestation and reforestation activities." From the U.S. Congress' Office of Technology Assessment (1991), "OTA also identified an energy conservation, energy supply, and forest management package that can achieve a 20 to 35 percent emissions reduction." From Shell Oil Company (Elliott and Booth 1990), "Biomass based power generation appears to have considerable potential both in the developed and developing world...The present indications are that such systems could be competitive in certain circumstances with today's price system." From the U.S. National Academy of Sciences (1991), "Action should be initiated now to slow and eventually halt tropical deforestation...Reforestation offers the potential of off-setting a large amount of CO_2 emissions." And, in a statement issued on behalf of President Bush to the first session of the U.N. International Negotiating Committee on Climate Change (1991), "Implementation of the President's Comprehensive Climate Change Strategy will result in United States greenhouse gas emissions in the year 2000 being equal to or below 1987 levels. The specific actions which will contribute to this result include...initiating a program to plant a billion trees a year and to make other forest improvements". Even McDonald's now has a corporate policy on tropical forests and climate change/forestry is fair and frequent game for political cartoonists.

With that somewhat eclectic introduction, let's take a quick look at the concerns about global climate change.

In 1958 David Keeling initiated a program of monitoring the atmospheric concentration of carbon dioxide in the earth's atmosphere. His measurements at Mauna Loa Observatory in Hawaii now provide us with a record that unambiguously documents a CO_2 increase from 315 ppm to 355 ppm over the ensuing 32 years (Keeling 1990). The Keeling record is supported by shorter time series from other stations, and measurements on tiny air bubbles extracted from drill cores in the polar ice sheets reveal that prior to the industrial revolution, the concentration was near 280 ppm (Barnola et al. 1987). During the last two centuries mankind has increasingly capitalized on the store of energy available in the earth as chemically reduced carbon in coal, oil, and natural gas. We now release to the atmosphere as CO_2 some 6 billion metric tons of carbon per year. Additional carbon is released, perhaps 1.5 billion metric tons per year, as forests are cleared and burned to provide agricultural land and living space for people (Dale et al. 1991). Patterns of CO_2 emissions and growth plus measurements of the stable isotopes of carbon strongly support the conclusion that the observed 25% growth in atmospheric CO_2 is indeed a consequence of man's activities on Earth (Watson et al. 1990).

We should not forget that very large quantities of carbon cycle continuously through terrestrial systems. Carbon is removed from the atmosphere by photosynthesis, returned to the atmosphere by plant and animal respiration, exchanged between the atmosphere and the surface ocean, etc. Figure 1 gives some idea of the magnitude and complexity of annual flows which are part of the natural carbon cycle and the relative magnitude of the perturbation caused by man. Although

man's contribution is still small by comparison, and will be damped out over the aeons, it is large enough to disturb the system over time scales of concern to us.
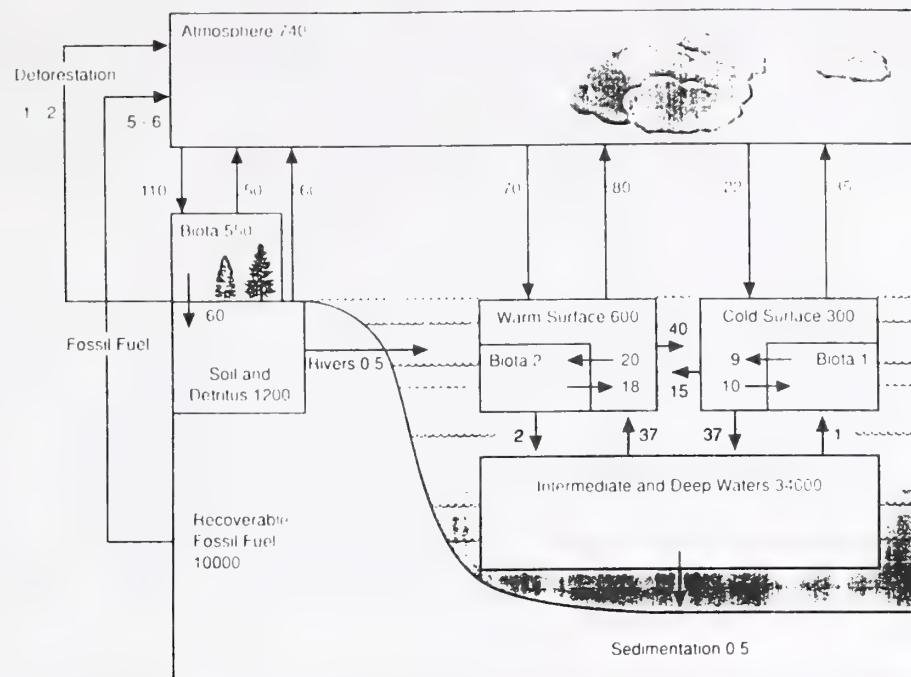
Figure 1: Schematic representation of the mass of carbon in major components of the global carbon cycle and the annual fluxes between components. All units are in 10^9 metric tons of carbon. (Adapted from Moore 1985 and Moore and Bolin 1986.)

The reason we worry about modest changes in the atmospheric concentration of a gas which occurs in the atmosphere at part-per-million levels has to do with its optical properties. The carbon dioxide molecule is inactive, and hence transparent, in the visible wavelengths at which the sun radiates to the earth, but has molecular vibration-rotation transitions in the infrared wavelengths at which the Earth radiates to space. The consequence of this is that increasing the CO_2 concentration causes an imbalance in the Earth's radiation budget and the build up of heat in the lower atmosphere. The "greenhouse effect" is real and well understood and explains why the mean Earth-surface temperature is near 15 ° C rather than at the calculated black-body temperature of -18 ° C. The question which confronts us now is the extent to which a change in the concentration of atmospheric greenhouse gases will change the Earth's climate system. A good part of the uncertainty has to do with the behavior of the Earth's hydrologic cycle. Very simply stated, increased heat at the Earth's surface can be expected to change the rate of evaporation and water is an important greenhouse gas. Also, once evaporation is increased, we have to be concerned with the effect on cloud cover. Clouds are very important to the Earth's radiation balance and their effect is dependent on cloud type and on their vertical and regional distribution. It is a complex system and our anticipation of the climatic impact of changes in atmospheric chemistry is thus dependent on mathematical models that try to simulate the climate system.

Detailed models of the Earth's climate system generally agree that an increase in atmospheric greenhouse gases will lead to an increase in the mean Earth-surface temperature. As an indication of scale, most models predict that a doubling of atmospheric CO_2 concentration would result in an increase in mean surface-air temperature of between 1.9 and 5.2 ° C (Mitchell et al. 1990). These

models have a very coarse grid size, typically on the order of 5 degrees of latitude and longitude, and have a difficult time predicting how temperature will change on a regional basis. The models have an even more difficult time predicting changes in other manifestations of climate, e.g. precipitation, and there are some very significant differences between models with respect to regional predictions (Grotch 1988). We are thus left with a general consensus that climate will change but with little useful information on how rapidly it will change or how these changes will be manifest at a specific locale. I may overstate this slightly to make a point (we do have some broad agreement on drying of continental interiors and greater change at higher latitude) but the point is that the parameters of greatest interest to farmers and foresters are the least reliably predicted.

Examination of historical records of climate may help some but it is very difficult to clearly establish cause and effect. In their recent assessment of the science, the Intergovernmental Panel on Climate Change (IPCC 1990) was willing to conclude that there has been a long-term increase in Earth-surface temperature, but they were unable to conclude that it was a consequence of greenhouse gas emissions: "Our judgement is that...global mean surface air temperature has increased by 0.3 to 0.6 ° C over the last 100 years...The size of this warming is broadly consistent with predictions of climate models, but it is also of the same magnitude as natural climate variability. Thus the observed increase could be largely due to this natural variability." The recent National Academy of Sciences study (1991) struggled with the same problems and concluded, "Despite the great uncertainties, warming is a potential threat sufficient to justify action now."

This discussion has focused, and will continue to focus, on CO_2 although there are a number of other gasses with increasing atmospheric concentrations and absorption spectra in the infrared wavelengths at which the Earth radiates energy to space. Methane, nitrous oxide, and the chlorofluorocarbons, for example, are of concern, although CO_2 is the most abundant and most important of the greenhouse gases. These gases vary in importance because of differences in absorption spectra, atmospheric lifetime, and ease with which their atmospheric increase might be controlled (see, for example, Shine et al. 1990). For changes in atmospheric chemistry which occurred during the decade of the 1980s, about half of the total potential to affect the Earth's radiation balance is attributable to the CO_2 (Ramanathan et al. 1987). Also, of course, CO_2 is the one greenhouse gas intimately linked with forests.

As noted earlier, the principal human activity responsible for current increases in atmospheric CO_2 is the burning of carbon-based fossil fuels. When fossil fuels are burned, carbon which has been long stored in the earth is released to the atmosphere. The burning of wood releases more CO_2 per unit of useful energy than does the burning of fossil fuels, but the implications for atmospheric CO_2 are fundamentally different. When wood is burned, carbon which was recently removed from the atmosphere via photosynthesis is simply returned to the atmosphere. So long as the tree is replaced by another tree, i.e. it is grown in a sustained-yield system, there is no net release of CO_2 to the atmosphere. To the extent that the tree is not replaced, there will be a net release of CO_2 from the biosphere, and herein lies our concern with forest clearing. We recognize, though, that when mature forest is harvested and replaced with young forest it may take a very long time to regain the carbon storage (Harmon et al. 1990). We should not conclude that there is no net CO_2 release from biomass fuel systems. For a current wood-fired power plant, for example, fossil fuels are used to plant, manage, harvest, and transport wood. Oxidation of these fuel "supplements" should be counted as CO_2 emissions required to operate the wood-fired system, even when there is no net emission from the wood combustion itself. As an example, Anthony Turhollow and I have examined the full accounts for producing ethanol from corn. We find that by the time corn is planted, fertilized,

harvested, and converted into ethanol; the oxidation of fossil fuel supplements has yielded CO_2 emissions equivalent to about 80% of the emissions from simply burning a quantity of gasoline of equal energy content (Marland and Turhollow 1991).

The total quantity of CO_2 released to the atmosphere from fossil fuel burning has now (1989) reached 5.97 billion metric tons of carbon per year, up from 1.64 billion tons in 1950. The U.S. share of this is 1.33 billion tons, approximately 5.4 tons of carbon per person per year (Marland 1990).

With broad agreement that the chemistry of the atmosphere is changing, that it is changing because of man's activities (particularly fossil fuel burning), and that this change bears some significant (but as yet poorly specified) risk of global and regional changes in climate; what, if anything, should we do? The current aphorism, especially in the U.S., is to pursue "no-regrets policies". The concept of no-regrets policies is that there are actions which have merit on other criteria, that we might responsibly pursue anyway, that would slow the rate of growth of greenhouse gases in the atmosphere. These are actions for which we would have no retrospective regrets even if the risks of climate change turn out to have been overstated. Some of the proposed measures would seek to limit the magnitude or rate of climate change while others would simply try to anticipate and accommodate the changes which occur. It is in this context that we return to the issues of forestry. Forests are perceived as being fundamentally "good" and most plans to confront global climate change include some effort to maintain and/or increase the amount of carbon which is stored in forests.

There are actually four basic questions for forestry in a global climate change context. First, if climate changes, how will it affect forests? Second, can we reduce CO_2 emissions by reducing the rate of destruction of forests, especially in the tropics? Third, can we remove carbon from the atmosphere and store it by increasing the area and/or carbon storage density in forests? And, fourth, can wood-based fuels from sustained-yield systems substitute for a significant fraction of fossil fuel usage? I don't wish to belabor details but perhaps I can provoke some useful thinking by bringing a variety of ideas together here.

Consider, first, the effect of climate change on forests. To begin with, we know that climate is but one of a number of stresses confronting forests. These stresses include ozone, acid precipitation, heavy metal deposition, and even the potentially beneficial direct effects of increasing ambient CO_2. We have data on tree seedlings to suggest that growth rate, drought tolerance, reproductive success, and other properties can be affected by increasing ambient CO_2 but there is little evidence to indicate how these will apply over the life of a tree or to complete ecosystems. It is species, not in-tact ecosystems, that will respond to climate change. The ability of trees to adapt to changes is different than for agricultural crops, for example, because of their longevity and long juvenile period (Brubaker 1986), and because of the intensity of management. On the other hand, trees are able to survive long periods of adverse conditions and they have a large genetic base for adaptation. Trees can also migrate under the pressure of changing climatic conditions. Evidence from the Holocene of eastern North America shows that tree species succeeded in migrating at 300 - 1000 meters per year as climate warmed behind retreating glaciers (Shugart et al. 1986). On the current earth, however, a distinct lack of ecosystem continuity could severely limit such migrations. Modeling studies by Al Solomon and his colleagues (e.g. Solomon 1986) suggest that the response to climate change in the Eastern United States will be a northward shift of forest zones, with expansion of forests into tundra areas in the north and losses of forest to non-forest vegetation on the southern and western margins. In his Mitchell Prize-winning essay, Daniel Botkin (1991),

"project(s) that global warming will lead to rapid and severe changes in forests of the Great Lakes States, with some areas suffering major die-backs during the first decades of the twenty-first century and some becoming deforested and unable to support trees by the end of that century." Botkin goes on to argue that the natural state of forests is, in fact, one of change and that we err in "believing that the natural condition is one of uniformity and constancy." As we discuss the options and opportunities below, we have to wonder how much the possibilities are amplified or constrained by the issues raised in this paragraph.

I don't want to devote much time here to a discussion of tropical forests except to note that the current annual clearing of tropical forests has been estimated to exceed the area of the state of Tennessee (e.g., Houghton et al. 1987; Myers 1990). The contribution to global CO_2 emissions is probably on the order of 25% of the total. With an integrated global economy, a well-mixed atmosphere, and one global pool of genetic material, we in the U.S. are immune from neither damage nor responsibility for what is happening in developing tropical nations. The area of U.S. forests decreased by 21 million hectares between 1953 and 1987 with loss of an additional 7 million hectares anticipated by 2010 (U.S. Congress 1991).

To illustrate the magnitude of the problem of offsetting fossil fuel related CO_2 emissions, consider the possibility of offsetting all 6 billion tons of carbon emissions with new forest. If we could establish new, fast-growing tree plantations on land that did not previously contain trees, and achieve a productivity of about 30 cubic meters equivalent in total biomass per hectare per year (i.e. a carbon uptake of 7.5 kg C per hectare per year), it would require 800 million hectares to accomplish a full offset. This is slightly smaller than the land area of Brazil. To offset emissions from a single coal-fired power plant operating at 38% thermal efficiency and with a capacity factor of 70% would require about 200 hectares of these plantations per megawatt of capacity. These rough calculations do not make allowance for the energy required to establish and maintain the plantation and they do not suggest what happens when the trees begin to mature and the growth rate drops off. They suggest that planting trees cannot solve the whole problem or even provide a permanent offset for a single fossil-fuel power plant. Trees could, however, provide a way to slow the growth of atmospheric CO_2 while we endeavor to either develop a more friendly energy system or establish a better understanding of the risks of climate change.

To contemplate tree-planting a little more broadly, consider Figure 2. The figure shows cumulative net emissions of CO_2 from a power plant as a function of time and suggests 4 scenarios. In scenario A, the current path, fossil fuels are being burned and there is a continuing increase in the cumulative amount of CO_2 discharged. In scenario B we envision that new forest is established so that growth of the forest is initially able to sequester an amount of carbon equivalent to that discharged by the power plant. As the forest matures, however, the rate of carbon uptake decreases until there is no net carbon uptake. Curve B then becomes parallel to curve A but offset from it by a quantity, a-b in the figure, equal to the amount of carbon stored in the mature forest. In scenario C, there is envisioned to be no fossil fuel burning and the power plant is fueled instead by harvesting from a mature forest. As mature forest is harvested, it is replaced by plantation forest which continues to provide fuel for the power plant. In the early stages, net CO_2 emissions closely track those from the coal-fired plant, but ultimately the rate of net emissions falls to zero as the plantation achieves a steady state of standing biomass. The carbon shown as c-o in the figure represents the difference in standing crop between the mature forest and the plantation forest. In scenario D, we envision that when construction of the power plant is initiated, there is simultaneous establishment of a plantation forest where forest did not previously exist. This plantation forest then provides fuel

for the power plant and some modest amount of carbon storage in standing biomass, d-o in the figure. Note that in the figure (c-o) + (d-o) = (a-b).
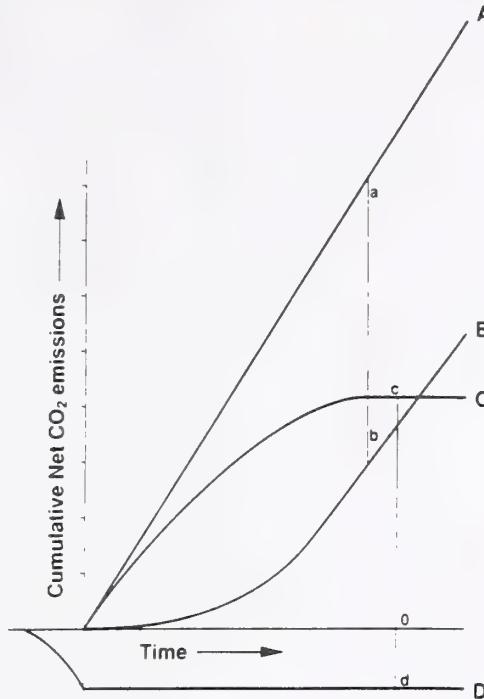
Figure 2: Qualitative representation of the net cumulative CO_2 emissions for a coal-fired power plant and associated forest. See text for a discussion of the four scenarios represented.

It is clear that the cartoon of Figure 2 leaves many unanswered questions because without quantification it tells us nothing about time, land area, productivity, standing crop, energy conversion efficiency, or cost. It doesn't distinguish between total and harvestable biomass. It does provide a framework for beginning to think seriously about these, and other, important variables. Whereas the figure suggests that the most attractive option, on net-CO_2-emissions criteria, should be the establishment of energy plantations where forest does not now exist, we know that the viability of energy plantations will depend on high yields, low energy inputs, low harvest costs, and high conversion efficiency. For biomass to provide a reasonable alternative to fossil fuels it is going to have to provide modern fuels (i.e. electricity or liquid transportation fuels) at reasonable cost. And, if biomass fuels are going to be advanced on environmental (global climate change) grounds, they are going to have to offer good environmental credentials on all fronts: habitat, soil loss, chemical inputs, nutrient cycling, combustion emissions, etc.

Scenario C above has not been a real possibility because we have not been able to burn wood with the same output of electricity per unit of carbon emitted as we can for coal. Recent work by Bob Williams (1990) and David Ostlie (1989) hold out the promise for highly efficient wood-combustion systems and work at the Solar Energy Research Institute and elsewhere is pursuing promising technologies for production of ethanol from cellulosic materials. I should emphasize, as an aside, that there may be advantages for woody crops, but at this point it is not clear why scenario D above should not be based, at least at some sites, on high-yield herbaceous crops rather than on trees.

Let me close with a brief quantitative summation of 3 recent studies and what they envision as possible or likely. Bob Moulton and Ken Richards (1990) of the U.S. Forest Service suggest that the U.S. could offset 56.4% of current CO_2 emissions with tree planting on "economically and environmentally marginal pasture and crop land and non-federal forest land." This would involve 140 million hectares, of which 30 million hectares are already forest land. The National Academy of Sciences (1991) takes a conservative approach in evaluating the Moulton and Richards data and suggests that a reasonable initial objective would be a 10% offset of current U.S. CO_2 emissions on 28.7 million hectares. The NAS study also considers replacing 2.4 quads (2.5×10^{18} joules) of fossil-fuel-fired electric power with biomass. The Office of Technology Assessment (U.S. Congress 1991) estimates that through a combination of planting trees on Conservation Reserve lands, increasing productivity, planting urban trees, general afforestation, and biomass energy the U.S. "might be able to offset about 2% of U.S. 1987 carbon emissions...in the year 2000 and 7.5% in 2015". They envision that economic opportunities for tree planting may exist on about 30 million hectares.

My conclusion is that where we can combine high yields with efficient harvest and conversion, energy crops should offer an attractive long-term contribution to reducing global emissions of CO_2. Where yields are lower and/or harvest more difficult, increasing forest area or improving forest management could provide a temporary brake on the growth in CO_2 emissions. In other areas, carbon storage may provide an added incentive to protect and preserve mature forests. The distinctions will depend on relative values of standing crop, achievable yield, and harvest cost. We are just beginning to get a realistic view of the possible magnitude of the contribution. The challenge is how to incorporate carbon fixation and storage as a management objective while maintaining a balance among other forestry management objectives.

LITERATURE CITED

America's Climate Change Strategy: An Action Agenda, 1991. A document published under the seal of the President of the United States for the first session of the Intergovernmental Negotiating Committee on Climate Change, Chantilly, Virginia, Feb. 4, 1991.

Barnola, J.M., D. Raynaud, Y. S. Korotkevich and C. Lorius, 1987. Vostok Ice Core Provides 160,000-year Record of Atmospheric CO_2, *Nature*, v. 329, p. 408-414.

Botkin, D.B., 1991. Global Warming and Forests of the Great Lakes States: An Example of the Use of Quantitative Projections in Policy Analysis. First prize paper, The George and Cynthia Mitchell International Prize for Sustainable Development, presented March 3-6, 1991, The 1991 Woodlands Conference, HARC Center for Growth Studies, The Woodlands, Texas.

Brubaker, L.B., 1986. Responses of Tree Populations to Climatic Change, *Vegetatio*, v. 67, p. 119-130.

Dale, V.H., R.A. Houghton, and C.A.S. Hall, 1991. Estimating the Effects of Land-use Change on Global Atmospheric CO_2 Concentrations, *Canadian J. Forest Res*, v. 21, p. 87-90.

Elliott, P. and R. Booth, 1990. Sustainable Biomass Energy, Selected Papers, Shell International Petroleum Company Ltd., London.

Grotch, S.L., 1988. Regional Intercomparisons of General Circulation Model Predictions and Historical Climate Data, DOE/NBB-0084, Office of Energy Research, U.S. Department of Energy.

Hansen, J.E., 1988. "The Greenhouse Effect: Impacts on Current Global Temperature and Regional Heat Waves." Testimony before the Committee on Energy and Natural Resources, U.S. Senate, 23 June, 1988.

Harmon, M.E., W.K. Ferrell, and J.F. Franklin, 1990. Effects on Carbon Storage of Conversion of Old-Growth Forests to Young Forests, *Science*, v. 247, p. 699-702.

Houghton, R.A., R.D. Boone, J.R. Fruci, J.E. Hobbie, J.M.Melillo, C.A. Palm, B.J. Peterson, G.R. Shaver, G.M. Woodwell, B. Moore, D.L. Skole, and N. Myers, 1987. The Flux of Carbon from Terrestrial Ecosystems to the Atmosphere in 1980 due to Changes in Land Use: Geographic Distribution of the Global Flux, *Tellus*, v. 39B, p. 122-139.

IPCC, 1990. Scientific Assessment of Climate Change, The Policymaker's Summary of the Report of Working Group I to the Intergovernmental Panel on Climate Change, World Meteorological Organization and U.N. Environment Programme.

Keeling, C.D., 1990. Atmospheric Carbon Dioxide Concentration, Mauna Loa, in T.A. Boden, P. Kanciruk, and M.P. Farrell, p. 9, Trends '90: A Compendium of Data on Global Change, ORNL/CDIAC-36, Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, Oak Ridge, TN, and personal communication.

Marland, G. and A. Turhollow, 1991. CO_2 Emissions from the Production and Combustion of Fuel Ethanol from Corn, *Energy - The International Journal*, in press.

Marland, G., 1990. Global and National CO_2 Emissions from Fossil Fuel Burning, Cement Production, and Gas Flaring, in T.A. Boden, P. Kanciruk, and M.P. Farrell (eds.) ORNL/CDIAC-36, p. 92-103, Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, Oak Ridge, TN.

Mitchell, J.F.B., S. Manabe, T. Tokioka, and V. Meleshko, 1990. Equilibrium Climate Change in J.T. Houghton, G.J. Jenkins, adn J.J. Ephraums (eds.), Climate Change: The IPCC Scientific Assessment, p. 131-172, University of Cambridge, University Press, Cambridge, U.K.

Moore, B., III, 1985. The Oceanic Sink for Excess Atmospheric Carbon Dioxide, in I.W. Duedall, D.R. Kester, and P.K. Park (eds.), Waters in the Ocean, v. 4, p. 95-125, J. Wiley & Sons, N.Y.

Moore, B., III, and B. Bolin, 1986. The Oceans, Carbon Dioxide, and Global Climate Change. *Oceanus*, v. 29, no. 4, p. 9-15.

Moulton, R.J. and K.R. Richards, 1990. Costs of Sequestering Carbon Through Tree Planting and Forest Management in the United States, U.S. Department of Agriculture Forest Service, General Technical Report WO-58.

Myers, N., 1990. Tropical Forests, in J. Leggett (ed.), Global Warming: The Greenpeace Report, p. 372-399, Oxford University Press, N.Y.

National Academy of Sciences, Committee on Science, Engineering, and Public Policy, 1991. Policy Implications of Greenhouse Warming, National Academy Press, Washington D.C.

Nairobi Declaration on Climatic Change, 1990. International Conference on Global Warming and Climatic Change: African Perspectives, 2-4 May, 1990, African Centre for Technology Studies, Nairobi, Kenya.

Noordwijk Declaration on Climate Change, 1989. Atmospheric Pollution and Climate Change, Ministerial Conference Held at Noordwijk, The Netherlands, on 6th and 7th November, 1989. (see U.S. IPCC News, v. 6, Dec., 1989, Office of International Activities, U.S. EPA and National Climate Program Office, U.S. NOAA).

Ostlie, L.D., 1989. The Whole Tree Burner: A New Technology in Power Generation, *Biologue*, Dec.-Jan., p. 7-9.

Ramanathan, V., L. Callis, R. Cess, J. Hansen, I. Isaksen, W. Kuhn, A. Lacis, F. Luther, J. Mahlman, R. Reck, and M. Schlesinger, 1987. Climate-Chemical Interactions and Effects of Changing Atmospheric Trace Gases, Reviews of Geophysics, v. 25, p. 1441-1482.

Sagan, C., 1989. Oral Presentation to the Global Change Conference sponsored by Cornell University and the National Governors' Association, February 28, 1989, N.Y., N.Y.

Shine, K.P., R.G. Derwent, D.J. Wuebbles, and J.-J. Morcrette, 1990. Radiactive Forcing of Climate, in J.T. Houghton, G.J. Jenkins, and J.J. Ephraums (eds.) Climate Change: The IPCC Scientific Assessment, p. 41-68, University of Cambridge, University Press, Cambridge, U.K.

Shugart, H.H., M.Y. Antonovsky, P.G. Jarvis, and A.P. Sandford, 1986. CO_2, Climatic Change and Forest Ecosystems, in B. Bolin, B.R. Doos, J. Jager, and R.A. Warrick (eds.), p. 475-521, Scope 29, The Greenhouse Effect, Climatic Change, and Ecosystems, John Wiley and Sons, N.Y.

Solomon, A.M, 1986. Transient Response of Forests to CO_2-Induced Climate Change: Simulation Modeling Experiments in Eastern North America, *Oecologia*, v. 68, p. 567-579.

U.S. Congress, Office of Technology Assessment, 1991. Changing by Degrees: Steps to Reduce Greenhouse Gases, OTA-0-482, Washington D.C., U.S. Government Printing Office.

U.S. Department of Agriculture, Forest Service, 1990. America the Beautiful: National Tree Planting Initiative.

Watson, R.T., H. Rodhe, H. Oeschger, and U. Siegenthaler, 1990. Greenhouse Gases and Aerosols, in J.T. Houghton, G.J. Jenkins and J.J. Ephraums (eds.) Climate Change: The IPCC Scientific Assessment, p. 1-40, University of Cambridge, University Press, Cambridge, U.K.

Williams, R.H., 1990. Biomass Gasifier/Gas Turbine Power and the Greenhouse Warming in Energy Technologies for Reducing Emissions of Greenhouse Gases, Proceedings of an Expert's Seminar, Paris, 12-14, April, 1989, Organization for Economic Cooperation and Development, International Energy Agency, Paris.

ADAPTING TO THE GREENHOUSE EFFECT
THROUGH TECHNOLOGICAL PREPAREDNESS

Peter Farnum
Weyerhaeuser Company
Tacoma, WA

While climatologists predict with some certainty that the rate of temperature change during the next 40 years will be greater than at any previous time in history, there is more certainty that the rate of technological change during this same time period will also be unprecedented. By adopting a strategy of technological preparedness the managers of forest plantations can reasonably expect to be able to develop trees and stands which are *adapted* to a greenhouse warmed world. Unfortunately, many aspects of this strategy are not available to those foresters who have stewardship over natural stands.

At Weyerhaeuser Company our strategy of technological preparedness is concentrated in the areas of silviculture, physiology, genetics, and biotechnology. We leave the development of the models needed to predict the impacts of the greenhouse effect to others. Important tactics in our strategy include: maintaining technological awareness, assessing the impact of the greenhouse on business decisions, evaluating threats to our forests, and adjustment of our *ongoing* research strategies.

For both our southern and western ownerships we are doing physiological research to understand and be able to screen our genetic families for characteristics which will enable them to be adapted to a greenhouse environment. It is important to note that this research was started long before there was any significant concern for the greenhouse effect. Once we know the important physiological characteristics and how to screen for them, we seek to increase their frequency in the populations which we manage. We anticipate that our ongoing programs in vegetative propagation both in rooted cuttings and in somatic embryogenesis will be useful in this regard. At some future date, once our biochemical understanding of tree physiology has advanced, we may be able to use the tools of genetic engineering to create greenhouse adapted trees.

Given an ongoing and goal oriented research program which has already been addressing key genetic and physiological characteristics of the tree populations under management, and given that this program will continue to develop and use knowledge and technological gains during the next 40 years, we believe that we can produce greenhouse adapted trees and stands through our strategy of technological preparedness. However, given the large complexity of biological systems, and the difficulty of predicting the unintended effects of new technologies, one who adopts this strategy must proceed with care and humility.

GENETIC VARIATION FOR AIR POLLUTION TOLERANCE

P. Berrang[1] and D.F. Karnosky[2]

Abstract.--Evidence of natural selection for air pollution
tolerance can indicate that populations of plants are being
affected by air quality. Data suggesting that ambient levels of
sulfur dioxide resulted in natural selection for tolerance to
this pollutant were published in the 1970s, but evidence for a
similar effect by ozone has been lacking. We have demonstrated
differences in apparent susceptibility to ozone among
populations of quaking aspen from areas that differ in air
quality. In several of our chamber fumigation studies,
populations from areas with relatively high concentrations of
ozone tended to show less injury than those from areas with
relatively low concentrations of ozone. Subsequent field studies
indicated that the clones with the most visible injury grew
slowest at doses of ozone typical of relatively polluted areas.
Quaking aspen is very intolerant of shade which suggests that
ambient levels of ozone in conjunction with intraspecific
competition is eliminating ozone-sensitive clones in areas with
relatively high levels of ozone.

INTRODUCTION

Man's activities are changing the chemical composition of the atmosphere
and will continue to do so in the future. These changes have the potential
to affect the growth and development of plants. Some of these changes can be
expected to result in natural selection for traits that confer resistance to
stresses they cause. We have chosen to restrict the scope of this discussion
to the effects of changes in sulfur dioxide and ozone concentrations. These
gases have always existed in the troposphere at some concentration, but their
levels today are determined largely by man's activities. These two gases
differ markedly in the pattern of changes in their concentration, in their
effects on the physiological processes, and in the scale at which they are
important.

SULFUR DIOXIDE
A HISTORICALLY IMPORTANT LOCAL POLLUTANT IN THE UNITED STATES

Smelters are the major source of sulfur dioxide, but coal-burning
electrical-generating facilities also produce significant quantities of this
pollutant (Heck 1984). Emissions of this pollutant in the United States more
than doubled between 1900 and the late 1930s and tripled by 1970. In 1970

1/ Biological Scientist, USDA Forest Service, Center for Forest Environmental
Studies, Dry Branch, Georgia

2/ Professor, School of Forestry and Wood Products, Michigan Technological
University, Houghton, Michigan

the U.S. Congress passed the original version of the Clean Air Act which initiated a major decrease in sulfur dioxide emissions. This decrease has been sustained for more than 20 years in spite of continued increases in coal consumption (NAPAP 1987).

Sulfur dioxide enters leaves of plants primarily through stomata. This pollutant causes relatively little damage as it passes through cell membranes (Mudd et al. 1984) and it is assumed that the primary mode of injury is through its affect on metabolic pathways inside the cell. There is no doubt that sulfur dioxide can reduce photosynthetic rates, but there is some question about the mechanism of this response (Hallgren 1984). Exposure to low concentrations of sulfur dioxide for a long time can have as much effect as exposure to a high concentration for a short time. Thus, evaluating the effects of an exposure to this, or any other pollutant requires knowing at least the concentration of the pollutant and the duration of the exposure. However, it is generally recognized that the exposures to sulfur dioxide that are required to cause foliar symptoms (e.g. Karnosky 1976) or growth reductions (e.g. Jensen and Dochinger 1979) in trees are considerably higher than those that occur in most rural parts of the United States today.

Primitive smelters once emitted large concentrations of sulfur dioxide close to the ground and caused catastrophic damage to natural populations. Early studies showed ecosystem impacts near these sources (Gordon and Gorham 1963, Scheffer and Hedgcock 1955), but smelters of this type have not existed in this country for many years. Up until the 1970s, a number of smaller point sources of sulfur dioxide existed in this country that, while they are not large enough to devastate the landscape, were large enough to cause foliar injury and reduced growth on some plants in their vicinity. Since the concentrations of sulfur dioxide being produced in the United States are now lower and spread over a much broader area, phytotoxic concentrations of this pollutant in its gaseous form are rare today. However, some excellent research was done on the genecological effects of sulfur dioxide in this country in the past and is still being conducted in some European countries today. In many cases comparable studies have not been conducted with ozone, so this work should be included in any discussion of the genecological effects of air pollutants.

If natural selection for air pollution tolerance has been occurring, populations in areas exposed to elevated levels of the pollutant should be more tolerant than populations in pristine areas. There is ample evidence to suggest that this is the case for herbaceous plants and sulfur dioxide tolerance. Taylor and Murdy (1975) showed that Carolina cranesbill (Geranium carolinianum L.) collected from populations within a kilometer of a large coal-fired electrical facility in Georgia were more tolerant of sulfur dioxide than plants collected from populations that were further away. Horsman et al. (1978) showed that perennial ryegrass (Lolium perenne L.) collected from populations in parts of the United Kingdom with high concentrations of sulfur dioxide were more tolerant of this pollutant than plants collected from populations in areas with lower concentrations of sulfur dioxide. Bell et al. (1982) obtained similar results for the effects of ambient levels of sulfur dioxide on a variety of grasses.

Although air pollution resistant genotypes can outcompete sensitive genotypes in a relatively polluted environment, they may be at a competitive disadvantage in a pristine environment. Wilson and Bell (1985) found that the frequency of sulfur dioxide-resistant genotypes in grasses increased significantly within four years after exposure to high concentrations of this pollutant were initiated. However, they also found that the frequency of sulfur dioxide-resistant genotypes started to decrease within three years of the time improvements were made in ambient air quality. Other researchers (Horsman et al. 1978) have not been able to verify these results.

The length of time between generations suggests that populations of trees would require more time to respond to air pollutants than populations of herbaceous plants. There are numerous reports that indicate there is substantial genetic variation for sulfur dioxide tolerance within populations of tree species (e.g. Bergman and Scholz 1985, Geburek et al. 1987, Karnosky 1977, and Karnosky and Steiner 1981). It is not unusual for researchers working with visible symptoms of sulfur dioxide injury to report that one clone or family shows five to ten times as much injury when exposed to the same amount of this pollutant as another clone or family from the same provenance. The large amount of genetic variation within populations coupled with the variation in ambient concentrations of sulfur dioxide around point sources suggests that there is potential for natural selection for sulfur dioxide tolerance in trees.

There is also a great deal of evidence to indicate that substantial genetic variation in sulfur dioxide tolerance exists among populations of tree species (e.g. Karnosky and Steiner 1981, Karpen 1970, Larsen et al. 1988, Oleksyn 1988, Vogl 1970). In spite of the extensive documentation of genetic variation for this trait, only a few researchers working with trees have reported evidence that suggests an association between sulfur dioxide tolerance of populations and ambient levels of this pollutant. Thor and Gall (1970) reported that when white pine Pinus strobus L. grown from seed collected near coal-burning steam plants were planted near point sources of sulfur dioxide, they appeared greener than those collected from other areas. Kriebel and Leben (1981) reported that white pine grown from seed collected in parts of the midwestern United States with presumably low levels of sulfur dioxide showed less injury than those collected in the eastern portions of the country when planted in polluted areas in Ohio.

A number of European researchers have evaluated genetic differences between sulfur dioxide resistant and sensitive individuals within tree species using isozyme analysis. Geburek et al (1987) found that within a single population of Scots pine (Pinus sylvestris L.), resistant individuals tended to be more heterozygous at a suite of nine electrophoretically detectable gene loci. Muller-Stark (1985) reported similar data for a population of European beech (Fagus sylvatica L.) showing extensive damage from an unknown stress. Bergman and Scholz (1985) reported that several alleles were rare, or in one case missing, from sulfur dioxide resistant individuals of Norway spruce (Picea abies (L.) Karst.) and suggested that certain rare alleles are being eliminated from natural populations by air pollutants.

OZONE
A REGIONAL POLLUTANT THAT AFFECTS PLANTS AT AMBIENT CONCENTRATIONS

Tropospheric ozone is a secondary pollutant. This means that it is not emitted directly by man's activities, but instead is formed in the lower atmosphere from other pollutants. Formation of this pollutant is a complex process, but involves nitrogen oxides and hydrocarbons reacting in the presence of sunlight to form ozone (Grennfelt and Schjoldager 1984). Nitrogen dioxides are produced primarily by electrical generating facilities and motor vehicles. The major man-made sources of hydrocarbons are transportation related (Heck 1984), but biogenic sources of these compounds, particularly conifers, produce two to three times as much hydrocarbons as anthropogenic sources in the United States. This has made it especially difficult to decrease ambient levels of ozone in the Southeast (Chameides 1988) where high temperatures, long growing seasons, and extensive pine forests predominate.

Because of the way ozone is formed, its spatial and temporal distribution is much different than that of sulfur dioxide. Since it is a secondary pollutant it is often formed some distance from where the primary pollutants are emitted. This tends to make ozone a problem in rural as well as urban areas (Lefohn and Jones 1986). Nationally, the highest concentrations of ozone are found in southern California, but elevated concentrations of ozone exist throughout much of the eastern United States (Vukovich et al. 1985). One consequence of the regional nature of this pollutant is that sites that differ significantly in ozone exposure are separated by long distances.

Ozone concentrations fluctuate annually and tend to be highest during the summer (Lefohn and Jones 1986). This is because more natural hydrocarbons are produced during the growing season and because temperatures are higher and sunlight is more intense. Ozone levels differ from day to day at any one location with the highest concentrations occurring when there are several consecutive days with high temperatures, intense sunlight, and stagnant air masses. At low elevations there is a great deal of diurnal fluctuation in ozone concentrations. Concentrations are relatively low in the morning, increase by a factor of three (or more) in early afternoon, and decrease gradually during the evening and night. These diurnal fluctuations are diminished at high elevations where ozone concentrations can remain high throughout the night (Berry 1964).

The degree of temporal fluctuation in ambient ozone concentrations makes it difficult characterize the amount of ozone that populations in different areas receive. The traditional method for describing ambient ozone levels is the growing season mean measured during either 7 hour (e.g. Heck et al 1984) or 12 hour days (e.g. Heagle et al. 1988). There is a limited amount of data (Musselman et al. 1983) that suggests the occasional high concentrations are more important in determining the response of plants to ozone than the seasonal mean concentration. For these reasons researchers often provide a measure of acute exposure to ozone; for example, the highest hourly average or the highest daily average (e.g. Heagle et al. 1988). More recently, Lefohn et al. (1988) have advocated the use of several other parameters such

as the number of occurrences above some critical concentration (e.g. 8 ppb), the total of ppm x hours for all concentrations above some critical concentration, or the sum of ppm x hours for all concentrations after weighting by a sigmoidal function that gives greater weights to the higher concentrations.

Since ozone enters leaves almost entirely through the stomata, stomatal conductance determines its rate of uptake (Reich 1987). The membranes of plant cells are one of the first sites of injury. Ozone has been shown to increase the permeability of both the plasma membrane and chloroplast membranes (Mudd et al. 1984). One of the first measurable responses to small increases in ozone levels are reductions in photosynthesis. Reich and Amundson (1985) reported decreases in rates of photosynthesis in several species of northern hardwoods and white pine. Sasek and Richardson (1989) have reported that rates of photosynthesis are also reduced in loblolly pine at twice the ambient concentrations of ozone in North Carolina. Dougherty and Teskey (personal communication from Phil Dougherty and Robert Teskey of the Un. of Georgia in 1990) have found reductions in photosynthesis in loblolly pine after two years exposure to ambient levels of ozone in Georgia.

These differences in photosynthesis can result in measurable decreases in growth, even under field conditions. Shafer and Heagle (1989) predicted using models constructed from data collected on loblolly pine (Pinus taeda L.) that the ambient levels of ozone in North Carolina would reduce growth rates by about 10% after two to three seasons of exposure. Wang et al. (1986) reported similar reductions in biomass of quaking aspen (Populus tremuloides Michx.) after three years exposure to ambient levels of ozone in New York State. In some cases the effect of ozone on the growth of plants is more evident on the root system than it is on the above-ground portion of the plant. This effect is especially important in perennial plants like trees (Cooley and Manning 1987).

The small size of some herbaceous plants makes it possible to expose entire communities to controlled doses of ozone. Researchers working with these plants have demonstrated that ambient levels of ozone can alter the species composition of communities. Rebbeck et al. (1988) showed that while clover (Trifolium repens L. c.v. Regal) dominated a field-grown pasture in North Carolina that was exposed to charcoal-filtered air, tall fescue (Fescue arundinacea Schreb. c.v. Kentucky 31) dominated the same pasture when it was exposed to ambient and above-ambient levels of ozone. Bennett and Runeckles (1977) reported similar results for a pasture in California. Demonstrating an ecological effect of ozone is considerably more difficult in forests because of the large size and perennial nature of the individual plants. The classic example of an ozone-induced ecological change in forests is the shift from ponderosa pine to white fir (Abies concolor Lindl. and Gord.) in southern California. Miller (1973) was able to document these effects, in part, because extremely high concentrations of ozone made visible symptoms of injury obvious. On some occasions the concentrations of ozone in these forests were more than four times the current National Ambient Air Quality Standard of 120 ppb. It has turned out to be considerably more difficult to demonstrate community-level changes in forests in other parts of the country where concentrations of ozone are much lower.

Ozone tolerance is known to be genetically controlled within a number of tree species. Karnosky (1977) demonstrated genetic differences in tolerance within populations of quaking aspen and Karnosky and Steiner (1981) reported differences within and among populations of green and white ash for this trait as well. A number of researchers have shown that ozone sensitivity is genetically controlled in loblolly pine (Adams et al. 1988, Horton et al. 1990, and Kress et al. 1982) and white pine (Berry 1973 and Houston and Stairs 1973).

ELIMINATION OF OZONE-SENSITIVE GENOTYPES EVIDENCE OF A LATENT EFFECT?

We feel that in some cases the more obvious ecological effects of ozone; for example, changes in species composition, are being masked by the subtle changes that can occur within populations of a single species. Data that show plants collected from populations in high ozone areas are more tolerant of this pollutant than plants collected from populations in pristine areas provide evidence to support this hypothesis. Dunn (1959) showed that lupine (Lupinus bicolor) grown from seed collected in Los Angeles had less foliar injury when grown in Los Angeles than lupine grown from seed collected in other areas. Although air quality monitoring data was not available for this study, ozone has historically been a major constituent of the smog in this city. We have conducted a series of experiments designed to determine whether this type of relationship exists for quaking aspen. This species was chosen because it is sensitive to ozone (Karnosky 1977), its natural distribution is large enough to include areas that differ in air quality, and it can be clonally propagated.

In 1983 we collected roots from 11 to 14 individuals in each of 5 natural populations of this species. These 5 populations were all in northeastern United States and included areas that differed in ambient ozone levels (Table 1). These root samples were clonally propagated and grown in a single greenhouse. The plants were exposed to 180 ppb ozone for 6 hours using an open-top chamber inside a greenhouse. One week after the fumigation we evaluated each plant for the percentage of leaves showing symptoms of ozone injury and the average surface area of the symptomatic leaves showing symptoms (Berrang et al. 1986).

Differences in ozone sensitivity among clones within populations were highly significant in all five populations. More importantly, there were large differences among populations as well. The average injury for clones was significantly less for the most polluted collection site than for the least polluted collection site and there was a negative correlation ($r = -0.98$) between average injury and ambient ozone levels (Table 1). We suggested that high ambient levels of ozone had eliminated ozone-sensitive clones from the population in the polluted area.

Other researchers offered a number of valid criticisms of this work. First, the ozone concentrations used were higher than those that commonly occur in northeastern United States and might not be relevant to field conditions. Second, since only a few collection sites were involved, the

Table 1 - Ambient ozone levels, average percentage of leaves injured, and ranges of clonal means for five populations of quaking aspen exposed to 180 ppb ozone for 6 hr in a chamber fumigation and to ambient air in a field planting near Millbrook, New York.

Population origin	Average ozone concentration	Percentage of leaves showing injury			
		Fumigation		Field	
		Population means	Range of clones	Population means	Range of clones
Cuyahoga Valley NRA Ohio	65 ppb	50_a	21-63	0_a	0-2
Saratoga NHP New York	49	58_{ab}	35-83	3_b	0-12
Acadia NP Maine	52	60_{ab}	22-84	4_b	0-18
Voyageurs NP Minnesota	26	71_{bc}	60-88	22_c	2-40
Isle Royale NP Michigan	14	75_c	58-88	23_c	0-48

Note: Numbers within columns are not significantly different at $P \leq 0.05$ as determined by Student-Newman-Keuls test for fumigation data or at $P \leq 0.1$ as determined by Dunn's non-parametric mean separation procedure for field data.

apparent association between ambient ozone and ozone tolerance might have been spurious. Third, the collection sites were widely separated and differences between sites in rainfall or temperature might be responsible for differences in ozone tolerance. Fourth, we did not show a relationship between visible injury and growth which should exist if ozone can eliminate sensitive clones. We have responded to each of these points in subsequent studies.

We established a plantation of this material about 100 km north of New York City in an area that experiences relatively high levels of ozone. There was considerably less injury in the field than there had been after fumigations, but the relative differences among the populations were the same (Table 1). This study also suggested that variation within populations was affected by ambient ozone. The amount of injury displayed by the least-injured (most tolerant) clone in each population was about equal, but there was a strong negative correlation ($r = -0.99$) between ozone concentrations at the population collection area and the the amount of injury displayed by the most-injured (least tolerant) clone (Berrang et al. 1989).

We conducted another fumigation experiment with clones from 15 populations of quaking aspen that spanned the entire United States (Table 2). The results were similar to our first study. Differences among

populations were statistically significant and ozone-tolerant populations were usually found in the areas with the highest maximum daily ozone concentrations. Populations from areas that met the Environmental Protection Agency's Ambient Air Quality Standard for ozone (i.e. had low levels of ozone) had significantly more injury than populations from areas that did not meet this standard. In spite of the close association between maximum daily ozone concentrations and visible injury, there was no relationship between seasonal average ozone concentrations and visible injury. Evaluation of the air quality data revealed that there was also no relationship between seasonal average ozone concentrations and maximum daily ozone concentrations. This suggests that there can be a very close relationship between the type of ozone exposure a population evolves under and the type of ozone exposure to which the population will be resistant (Berrang et al. 1991).

We also found an association between climatic variables and ozone tolerance in this study using rank correlation. Leaf injury was negatively correlated with both minimum annual temperature ($r = -0.66$, $P = 0.01$) and annual precipitation ($r = -0.50$, $P = 0.06$). Perhaps the large amount of precipitation at Crater Lake helps explain why this population was more ozone tolerant than would be expected based on the presumably low levels of ambient ozone at its origin. This relationship between climatic variables and injury does not negate the relationship between ozone and injury. Partial correlation revealed there was a significant relationship between ambient ozone and some measures of ozone injury even when the effects of precipitation and temperature are taken into account (Berrang et al. 1991).

There seems to be a good relationship between visible injury determined under our test conditions and growth in the field for this species. Two sensitive clones and one resistant clone from Karnosky's 1977 study of genetic differences in ozone tolerance were established in the same field in New York described earlier. After ten years growth in this high ozone environment, the resistant clones were about twice as as tall as the sensitive clones (Berrang et al. 1989). Eighteen clones identified as sensitive and 18 identified as tolerant based on visible injury were established in open-top chambers in the field in Michigan in 1989. When exposed to chronic levels of ozone (80 ppb for 6 h/day for 3 days/wk) there was no reduction in stem biomass for the ozone-tolerant clones, but the growth of ozone-sensitive clones was reduced by 46%. It is also interesting to note that the sensitive clones outgrew the tolerant clones in charcoal-filtered air, suggesting that there may be a cost associated with ozone tolerance that makes these clones less competitive in a pristine environment (Karnosky and Scholz 1990).

Ambient levels of ozone are probably not high enough to kill even the most ozone-sensitive clones we tested. However, quaking aspen is extremely sensitive to shading (Baker 1949 and Shirley 1941), and differential growth rates caused by ozone in conjunction with competition could result in the elimination of ozone sensitive clones from populations in polluted areas. The correlations we have reported between ambient levels of ozone and ozone tolerance suggests that an early stage of selection, the elimination of sensitive genotypes, has begun to occur in some North American forests.

Table 2 - Ambient ozone levels, average percentage of leaves injured, and range of clonal means for 15 populations of quaking aspen exposed to 150 ppb ozone for 6 hours in a chamber fumigation.

Population origin	Average ozone concentration	Average maximum daily ozone concentration	Percentage of leaves showing injury	
			Population mean	Range of clones
Apostle Islands NL Wisconsin	na	na	54 cd	22-85
Crater Lake NP and Winema NF, Oregon	na	na	34 a	10-64
Cuyahoga Valley NRA Ohio	31	118	41 abc	29-65
Delaware Water Gap NRA Pennsylvania	32	136	41 abc	14-65
Glacier NP Montana	na	na	64 d	18-91
Indiana Dunes NL Indiana	31	126	34 a	21-57
Monongahela NF and Blackwater Falls SP, West Virginia	45	116	43 abc	23-63
Rocky Mountain NP Colorado	45	83	55 cd	29-82
Saguaro NM and Coronado NF Arizona	38	75	35 a	18-54
Sequoia NP and Sequoia NF California	48	106	38 ab	14-65
Sleeping Bear Dunes NL Michigan	na	na	41 abc	14-60
Voyageurs NP Minnesota	29	77	51 bcd	22-80
Wind Cave NP, Mt Rushmore NM, and Blackhills NF South Dakota	34	63	53 cd	20-72
Yellowstone NP Wyoming	37	62	59 d	30-80
Yosemite National Park California	34	94	43 abc	24-68

Note: Numbers within columns are not significantly different at $P \leq 0.05$ as determined by Student-Newman-Keuls test.

LITERATURE CITED

Adams, M.B., Kelly, J.M., and Edwards, N.T. 1988. Growth of Pinus taeda L. seedlings varies with family and ozone exposure level. Water, Air and Soil Pollut. 38:137-150.

Baker, F.S. 1949. A revised tolerance table. J. For. 47:179-181.

Bell, J.N.B., Ayazloo, M. and Wilson, G.B. 1982. Selection for sulphur dioxide tolerance in grass populations in polluted areas. In Urban Ecology. Edited by R. Bornkamm, J.A. Lee, and M.R.D. Seaward. Blackwell Scientific Pubs., Oxford. p 171-180.

Bennett, J.P. and Runeckles, V.C. 1977. Effects of low levels of ozone on plant competition. J. Appl Ecol. 14:877-880.

Bergmann, F. and Scholz, F. 1985. Effects of selection pressure by SO_2 pollution on genetic structures of Norway spruce (Picea abies). In: Population Genetics in Forestry, H.-R. Gregorius, Lecture Notes in Biomathematics, Springer, Berlin. p 267-275.

Berrang, P., Karnosky, D.F., Mickler, R.A. and Bennett, J.P. 1986. Natural selection for ozone tolerance in Populus tremuloides. Can. J. For. Res. 16:1214-1216.

Berrang, P., Karnosky, D.F., and Bennett, J.P. 1989. Natural selection for ozone tolerance in Populus tremuloides: field verification. Can. J. For. Res. 19:519-522.

Berrang, P., Karnosky, D.F., and Bennett, J.P. 1991. Natural selection for ozone tolerance in Populus tremuloides: an evaluation of nationwide trends. Can. J. For. Res. 21:(in press).

Berry, C.R. 1964. Differences in concentrations of surface oxidant between valley and mountaintop conditions in the southern Appalachians. J. Air Pollution Control Assoc. 14:238-239.

Berry, C.R. 1973. The differential sensitivity of eastern white pine to three types of air pollution. Can. J. For. Res. 3:543-547.

Chameides, W.L., Lindsay, R.W., Richardson, J., and Kiang, C.S. 1988. The role of biogenic emissions in urban photochemical smog: Atlanta as a case study. Science 241:1473-1475.

Cooley, D.R. and W.J. Manning. 1987. The impact of ozone on assimilate partitioning in plants: a review. Environ. Pollut. 47:95-113.

Dunn, D.B. 1959. Some effects of air pollution on Lupinus in the Los Angeles area. Ecology 40:621-625.

Geburek, T, Scholz, F., Knabe W., and Vornweg, A. 1987. Genetic studies by
    isozyme gene loci on tolerance and sensitivity in an air polluted Pinus
    sylvestris field trial. Silvae Genetica 36:49-53.

Gordon, A.G. and Gorham, E. 1963. Ecological effects of air pollution from
    an iron-sintering plant at Wawa, Ontario. Can. J. Bot. 41:1063-1078.

Grennfelt, P. and Schjoldager, J. 1984. A mounting menace. Ambio 13:61-67.

Hallgren, J.-E. 1984. Photosynthetic gas exchange in leaves affected by air
    pollutants. In: Gaseous Air Pollutants and Plant Metabolism, M.J. Koziol
    and F.R. Whatley (eds.), Butterworths, Boston. p 147-158.

Heagle, A.S., Miller, J.E., Heck, W.W., and Patterson, R.P. 1988. Injury
    and yield response of cotton to chronic doses of ozone and soil moisture
    deficit. J. Environ. Qual. 17:627-635.

Heck, W.W. 1984. Defining gaseous pollution problems in North America.
    In: Gaseous Air Pollutants and Plant Metabolism, M.J. Koziol and F.R.
    Whatley (eds.), Butterworths, Boston. p 35-48.

Heck, W.W., Cure, W.W., Rawlings, J.O., Zaragoza, L.J., Heagle, A.S.
    Heggestad, H.E. Kohut, R.J. Kress, L.W., and Temple P.J. 1984. Assessing
    Impacts of ozone on agricultural crops: II. Crop yield functions and
    alternative exposure statistics. J. Air Pollution Control Assoc.
    34:810-817.

Horsman, D.C., Roberts, T.M., and Bradshaw, A.D. 1978. Evolution of
    sulphur dioxide tolerance in perennial ryegrass. Nature 276:493-494.

Horton, S.J., Reinert, R.A., and Heck W.W. 1990. Effects of ozone on three
    open-pollinated families of Pinus taeda L. grown in two substrates.
    Environ. Pollut. 65:279-292.

Houston, D.B. and Stairs, G.R. 1973. Genetic selection of sulfur dioxide
    ozone tolerance in eastern white pine. For. Sci. 19:267-271.

Jensen, K.F. and Dochinger, L.S. 1979. Growth responses of woody species
    to long-term and short-term fumigation with sulfur dioxide. USDA Forest
    Service Res. Pap. NE-442.

Karnosky, D.F. 1976. Threshold levels for foliar injury to Populus
    tremuloides by sulfur dioxide and ozone. Can. J. For. Res. 6:166-169.

Karnosky, D.F. 1977. Evidence for gentic control of response to sulfur
    dioxide and ozone in Populus tremuloides. Can J. For. Res. 7:437-440.

Karnosky, D.F. and Scholz, F. 1990. Genetic implications of air pollution for
    forestry at present and in the future. Proc. 19th IUFRO World Congress,
    Aug. 5-11, Montreal. (in press).

28

Karnosky, D.F. and Steiner, K.C. 1981. Provenance and family variation in response of Fraxinus americana and F. pennsylvanica to ozone and sulfur dioxide. Phytopathology 71:804-807.

Karpen, D.N. 1970. Ozone and sulfur dioxide synergism: foliar injury to a ponderosa pine geographic race plantation in the Pugent Sound region. Plant Dis. Reporter 54:945-948.

Kress, L.W., Skelly, J.M., and Hinkelmann, K.H. 1982. Relative sensitivity of 18 full-sib families of Pinus taeda to O_3. Can. J. For. Res. 12:203-209.

Kriebel, H.B. and Leben, C. 1981. The impact of SO_2 air pollution on the gene pool of eastern white pine. Proceedings of the IUFRO World Congress, Division 2, Kyoto, Japan. p. 185-189.

Larson, J.B., Qian, X.M., Scholz, F. and Wagner, I. 1988. Ecophysiological reactions of different provenances of European silver fir (Abies alba Mill.) to SO_2 exposure during winter. Eur. J. For. Pathol. 18:44-50.

Lefohn, A.S. and C.K. Jones. 1986. The characterization of ozone and sulfur dioxide air quality data for assessing possible vegetation effects. J. Air Pollution Control Assoc. 36:1123-1129.

Lefohn, A.S., Laurence, J.A. and R.J. Kohut. 1988. A comparison of indices that describe the relationship between exposure to ozone and reduction in the yield of agricultural crops. Atmospheric Environm. 22:1229-1240.

Miller, P.L. 1973. Oxidant-induced community change in a mixed conifer forest In: Air Pollution Damage to Vegetation. Advan. Chem. Ser. 122:101-117.

Mudd, J.B., Banerjee, S.K., Dooley, M.M. and Knight, K.L. 1984. In: Gaseous Air Pollutants and Plant Metabolism, M.J. Koziol and F.R. Whatley (eds.), Butterworths, Boston. p 105-116.

Muller-Stark, G. 1985. Genetic differences between "tolerant" and "sensitive" beeches (Fagus sylvestris L.) in an environmentally stressed adult forest stand. Silvae Genetica 34:241-247.

Musselman. R.C., Oshima, R.J., and Gallavan, R.E. 1983. Significance of pollutant concentration distribution in the response of 'red kidney' beans to ozone. J. Amer. Soc. Hort. Sci. 108:374-351.

National Acid Precipitation Assessment Program (NAPAP). 1987. Interim assessment: the casues and effects of acidic deposition. C.N. Herrick (ed.). National Acid Precipitation Assessment Program, Vol.1, Executive Summary. Office of Director of Research, Washington, D.C.

Oleksyn, J. 1988. Height growth of different European Scots pine Pinus sylvestris L. provenances in a heavily polluted and a control environment. Environmental Pollution 55:289-299.

Rebbeck, J., Blum U., and Heagle, A.S. 1988. Effects of ozone on the regrowth and energy reserves of a ladino clover-tall fescue pasture. J. Appl. Ecol. 25:659-681.

Reich, P.B. 1987. Quantifying plant response to ozone: a unifying theory. Tree Physiology 3:63-91.

Reich, P.B. and Amundson, R.G. 1985. Ambient levels of ozone reduce net photosynthesis in tree and crop species. Science 230:566-570.

Sasek, T.W. and C.J. Richardson. 1989. Effects of chronic doses of ozone on loblolly pine: photosynthetic characteristics in the third growing season. For. Sci. 35:745-755.

Shafer, S.R. and A.S. Heagle. 1989. Growth responses of field-grown loblolly pine to chronic doses of ozone during multiple growing seasons. Can. J. For. Res. 19:821-831.

Scheffer, T.C. and Hedgecock, G.G. 1955. Injury to northwestern forest trees by sulfur dioxide from smelters. USDA Tech Bull 1117.

Shirley, H.L. 1941. Restoring conifers to the Lake States. Tech. Bull. U.S. Dep. Agric. No. 763.

Taylor, G.E., Jr. and Murdy, W.H. 1975. Population differentiations of an annual plant species, Geranium carolinianum, in response to sulfur dioxide. Bot. Gaz. (Chicago), 136:212-215.

Thor, E. and Gall, W.R. 1978. Variation in air pollution tolerance and growth rate among progenies of southern Appalachian white pine. Metropolitan Tree Improvement Alliance Proc. 1:80-86.

Vukovich, F.M., Fishman, J., and Browell, E.V. 1985. The reservoir of ozone in the boundary layer of the eastern United States and its potential impact on the global tropospheric ozone budget. J. Geophysical Res. 90(D3):5687-5698.

Wang, D., Karnosky, D.F., and Borman, F.H. 1986. Effects of ambient ozone on the productivity of Populus tremuloides Michx. grown under field conditions. Can. J. For. Res. 16:47-55.

Wilson, G.B. and J.N.B. Bell. 1985. Studies on the tolerance to SO_2 of grass populations in polluted areas. III. Investigations on the rate of development of tolerance. New Phytol. 100:63-77.

RESPONSE OF LOBLOLLY PINE SEEDLING GENETIC VARIATION TO OZONE

A. E. Wiselogel [1]

Abstract.--Ozone was observed to be a significant source of variation for height and diameter relative growth rates and percent visible foliar injury for 30 open pollinated loblolly pine families. The relative height growth rates contained a significant family by ozone interaction. Relative diameter growth rates had a significant family variation. There were no significant family effects observed for percent visible foliar injury. The change in relative height growth rate additive genetic variance and narrow sense heritability over increased ozone dosage indicated the presence of genetic control. Relative diameter growth rate additive genetic variance and heritability remained constant across increased ozone dosage. Thus, there were no indications of genetic control over the response of diameter relative growth rate to ozone. The lack of significant family variation in percent visible foliar injury indicated the lack of observable genetic control over this variable.

Keywords: Pinus taeda L., narrow sense heritability, phenotypic variation, dose response.

INTRODUCTION

Over the past decade an extensive research effort to determine the environmental impacts of pollution was sponsored by the United States government. Concerns over the effects of pollution from anthropogenic sources on forest ecosystems was addressed by the Forest Response Program (FRP). Long-term trends of ambient ozone in the southeastern United States suggested that ozone concentrations during the growing season often occur at levels sufficient enough to cause injury to plants (Pinkerton and Lefond 1987). A 1985 U. S. Forest Service report indicated that pines in the Georgia and South Carolina Piedmont region may have been in a growth decline. One of several possible causal agents mentioned in that report was ozone (Sheffeild et al. 1985). Ozone differs from most air pollutants in that it is a regionwide pollutant and not usually associated directly with a particular point source (Pinkerton and Lefond 1987). This fact led to including of ozone, with acid rain, in government sponsered studies involving southern pines.

---

[1] Senior Scientist, Chemical Conversion Division, Solar Energy Research Institute, Golden, Colorado. The reported work was performed while the author was at Texas A&M University.

The detrimental effect of ozone on loblolly pine physiology and above grown growth has been established (Pye 1988, Reich 1987). Ozone enters the pine foliage through the stomates (Tingy and Hogsett 1985, Olsyzk and Tingy 1982). In the mesophyll layer inside the foliage ozone oxidizes cellular membranes (Heath 1980). The injury to the interior portions of the foliage can cause a reduction in net photosynthetic rates and/or an increase in respiration (Sasek and Richardson 1989, Hanson et al. 1988, Reich 1987). With less carbon inputs loblolly pine seedlings have been reported to change normal carbohydrate allocation patterns. Several studies reported that carbohydrates accumulate in the above ground portion of the seedling. Thus, the root to shoot ratios can change significantly due to ozone exposure (Wiselogel et al. 1990, Shafer and Heagle 1989, Pye 1988). A change in the root to shoot ration may predispose the seedling to drought or nutrient stress.

Several studies in Europe have reported the effect of genetic diversity on how forest trees respond to air pollution (Bergmann and Scholz 1987, Geburke et al. 1987). These studies employed isozymes to describe the genetic variation in air pollution resistance and susceptible tree populations. However, response to air pollution was characterized as visible foliar injury and not changes in physiological processes or reduction in growth. Studies with loblolly pine show a low correlation between visible foliar injury and growth reduction (Wiselogel et al. 1991, Adams et al. 1988, Pye 1988). Several reports on loblolly pine response to ozone have indicated the existence of genetic control (Wiselogel et al. 1991, Shafer and Heagle 1989, Adams et al. 1988). Shafer and Heagle (1989) reported a family by ozone interactions for loblolly pine seedling growth variables after 3 years of ozone treatments. Adams et al. (1988) observed similar results after one season of fumigation. The observed significant family by ozone interaction indicated that families perform differently as ozone concentrations increased. This trend is indicative expression of additive genetic control over the observed response.

A study by Wiselogel et al. (1991) observed family by ozone interactions for relative height growth rate and above ground biomass. This study was designed to screen the response of 30 commercially available open pollinated loblolly pine families to ozone induced stress. While not specifically designed for precise estimation of genetic variation the study does allow for its calculation. Thus, the study provided an opportunity to observe changes in genetic variation over increasing ozone concentrations.

METHODS

The study used 22-week-old seedlings from 30 open pollinated loblolly pine families. Prior to ozone treatments the seedlings had been grown in a charcoal-filtered greenhouse. Square wave dispensing of ozone in fumigation chambers occurred for 8 hours a day, 4 consecutive days a week, for 9 weeks. Ozone treatments of charcoal-filtered or $> .01$ ppm, .16 ppm, and .32 ppm were applied during the study. The ozone treatments resulted in ozone dosage exposures of ~ 1, 46, and 92 ppm hours^{-1}. The study ozone dosages are comparable to ambient levels observed for the southeastern United States (Pinkerton and Lefond 1987). While the ozone exposures were of an artificial acute nature rather than a more natural chronic, there is evidence that both types of exposures have similar adverse effects on tree growth (Pye 1988, Reich 1987).

Due to the number of available fumigation chambers the experiment was design as a nested factorial with fumigation chamber effects confound in ozone treatments. To reduce the impact of confounded chamber effects on ozone treatments, seedlings and their ozone treatments were rotated to each chamber. Thus, each treatment was applied in every chamber an equal length of time. There were four replications within each ozone treatment. The replications were designed to block out position effects within the chambers. For a more detailed description of the study methodology see Wiselogel et al. (1991).

An analysis of variance (ANOVA) using PROC ANOVA (SAS 1982) was performed to determine ozone and family effects on monitored variables. Based on the results, relative height and diameter growth rate, and percent visible foliar injury were selected for genetic analysis. All sources of variation were considered random. Since the number of seedlings per family was small, the data was studentized to keep the narrow sense individual heritabilities below 1. Estimated mean squares were used to calculate phenotypic, genetic, and environmental variances for the variables of interest (SAS 1982). The open pollinated families were considered half-sib families to facilitate the calculation of heritabilities (Zobel and Talbert 1984).

Percent visible foliar injury (PVFI) was assessed on each seedling at the end of the study. PVFI was based on the proportion of the total foliar area which was chlorotic or necrotic. The percentage data was transformed with arcsin transformation as recommended by Snedecor and Cochran (1967) for binomial proportions. Relative growth rate for diameter and height was calculated as the difference between the natural log of the variable at the end and beginning of the study divided by the number of weeks in the study (Kramer and Kozlowski 1979).

RESULTS AND DISCUSSION

PVFI significantly increased with increased ozone dosage (Table 1). Chlorotic mottling and tip-burn was observed in the .32 ppm ozone treatment after 3 weeks and in the .16 ppm ozone treatment after 6 weeks.

Table 1. The probability of obtaining a greater f-test for percent visible foliar injury, relative diameter growth rate, and relative height growth rate of loblolly pine seedlings exposed to ~ 1, 46, and 92 ppm hours^{-1} ozone over 9 weeks.

Source of Variation	Percent Visible Foliar Injury	Relative Diameter Growth Rate	Relative Height Growth Rate
Ozone	0.0153	0.0787	0.0012
Family	0.1535	0.0001	0.0001
Family x Ozone	0.8788	0.3264	0.0064

The visible injury for seedlings in the ~1 ppm hours^{-1} ozone treatment consisted of necrosis of primary needles and foliage injured during chamber rotations. At 31 weeks of age, mature secondary foliage was dominant in all seedlings and the juvenile primary foliage had or was senescent. Seedlings exposed to 46 ppm hours^{-1} of ozone exhibited a combination of chlorosis and tip-burn with only a few seedlings having just one or the other. All seedlings exposed to 92 ppm hours^{-1} of ozone had both chlorosis and tip-burn.

There were no significant family or family by ozone variation observed for PVFI. This indicated that PVFI is a poor variable for assessing genetic effects on loblolly pine seedling response to ozone. All open pollinated family relative performance was the same for each ozone treatment.

A linear increase in PVFI occurred with increased ozone dosage (Figure 1). As PVFI increased the proportion of genetic and environmental variation in the phenotypic variation changed. At the low ozone treatment, narrow sense individual heritability (h^2) was observed to be 0.7. The high h^2 could have resulted from genetic control over the senescence of primary foliage. As ozone dosage increased so did phenotypic and environmental variation. The constant level of genetic variation resulted in a rapid decrease in h^2 of PVFI.

Figure 1. The mean response of percent visible foliar injury, phenotypic variation (Vp), additive genetic variation (Vg), environmental variation (Ve), and narrow sense individual heritabilities to ozone.
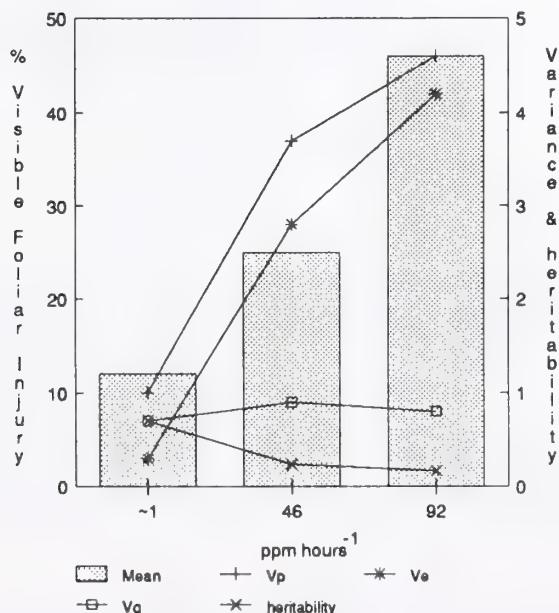
Relative diameter growth rate (RDGR) was not as responsive to ozone treatments as PVFI. The results of the ANOVA suggested that the majority of variation observed for RDGR was located among families (Table 1). These family differences remained constant across increased ozone dosage as indicated by the non-significant family by ozone interaction.

The reduction of RDGR by ozone was not linear in respect to dosage (Figure 2). There

was essentially no difference between the response of loblolly pine seedlings to 46 and 92 ppm hours[-1] ozone. With the increased ozone dosage, the phenotypic and genetic variation for RDGR increased by 20% while the environmental variation remained constant. This response resulted in a slightly increased h^2. The change in h^2 did not result in a change of family performance, thus, there is no indication of genetic control over RDGR response to ozone. The families with the highest RDGR under pristine conditions had the highest growth rate under high ozone conditions.

Figure 2. The mean response of relative diameter growth rate, phenotypic variation (Vp), additive genetic variation (Vg), environmental variation (Ve), and narrow sense individual heritabilities to ozone.
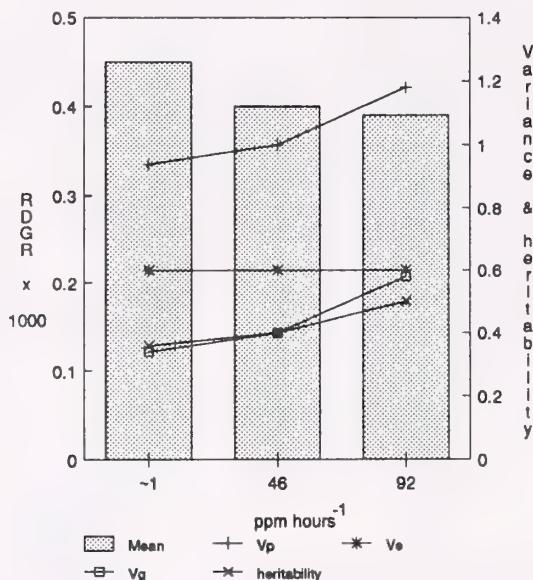
All sources of variation were significant for relative height growth rate (RHGR) (Table 1). As ozone dosage increased open pollinated families responded differently. The resultant significant family by ozone interaction indicated that genetic control existed over the response of RHGR to ozone. RHGR decreased with increased ozone dosage (Figure 3). The phenotypic variation remained constant as ozone dosage increased, however, genetic variation increased and environmental variation decreased. As a result, h^2 increased with ozone dosage. The change in h^2 supported the existence of genetic control over RHGR response to ozone as implied by the family by ozone interaction observed in the ANOVA.

Of the 30 open pollinated families used in the study, 22 families had a non-significant decrease in RHGR with increased ozone dosage (Figure 4). Seven families had a significant decrease in RHGR with increased ozone dosage, and 1 family increased RHGR at 92 ppm hours[-1] ozone. Family 8-80 was among the slowest growing families for ~1 and 46 ppm hours[-1] ozone and intermediate in RHGR for 92 ppm hours[-1] ozone. The significant change in performance for 8 of the 30 open pollinated families indicated that genetically endowed responce to ozone existed for the RHGR. The degree to which ozone effects tree height at time of evaluation for progeny test will determine if commercial populations in areas with high ambient

ozone are selected for ozone resistance.

Figure 3. The mean response of relative height growth rate, phenotypic variation (Vp), additive genetic variation (Vg), environmental variation (Ve), and individual narrow sense heritabilities to ozone.
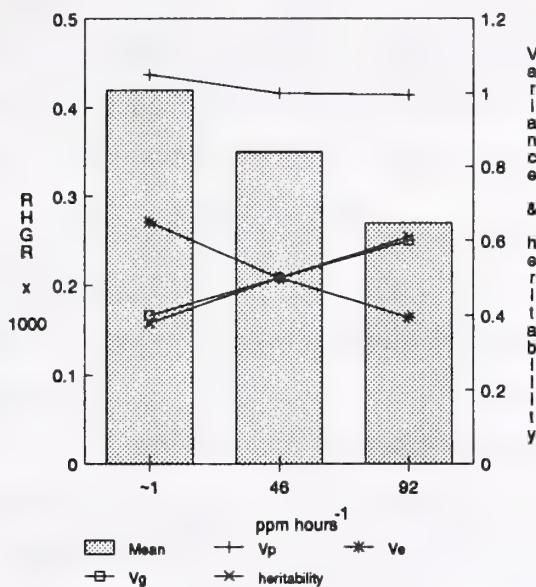
Figure 4. The relative height growth response of the 22 non-significantly effected families, 8 susceptible families, and the resistant family to ozone.
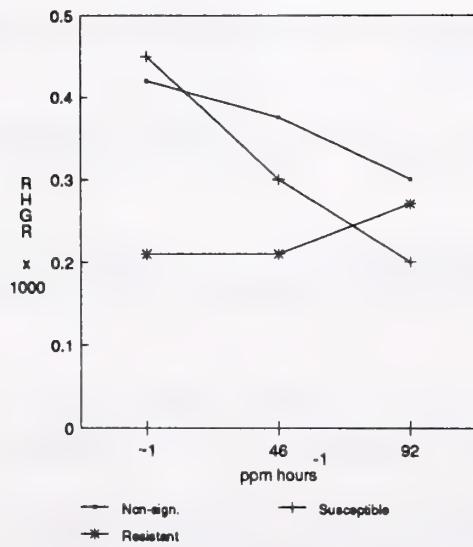
CONCLUSIONS

The use of percent visible foliar injury as a measure of loblolly pine response to ozone is tenuous. This study provided no evidence of genetic control over the visible foliar injury response to ozone. Relative diameter growth rate was the least sensitive of the response variables to ozone. The relative rankings of open pollinated family performance were not affected by increased ozone dosage. Relative height growth rate provided evidence of genetic control over the seedlings response to ozone dosage. If this fact is true, then it could be possible that loblolly pine families may have been selected or rogued because of their genetic response to ozone.

LITERATURE CITED

Adams,M.B., J.M.Kelly, and N.T.Edwards. 1988. Growth of Pinus taeda L. seedlings varies with family and ozone exposure level. Water, Air, and Soil Pollution 38: 137-150.

Bergmann,F., and F.Scholz. 1987. The impact of air pollution on the genetic structure of norway spruce. Silvae Genetica 36,2: 80-83.

Geburek,Th., F.Scholz, W.Knabe, and A.Vornweg. 1987. Genetic studies by isozyme gene loci on tolerance and sensitivity in an air polluted Pinus sylvestris field trial. Silvae Genetica 36,2: 49-60.

Hanson,P.J., S.B.McLaughlin, and N.T.Edwards. 1988. Net CO_2 Exchange of Pinus taeda shoots exposed to variable ozone levels and rain chemistries in field and laboratory settings. Physiologia Plantarum 74: 635-642.

Heath,R.L. 1980. Initial events in injury to plants by air pollutants. Annual Review of Plant Physiology 31: 395-431.

Kramer,P.J., and T.T.Kozlowski. 1979. Physiology of Woody Plants. Academic Press. NY, NY. 111 p.

Kress,L.W., H.L.Allen, J.E.Mudand, and W.W.Heck. 1988. Response of loblolly pine to acidic precipitation and ozone. P88-70.5 in Air Pollution Control Association Meetings, Dallas, TX.

Olszyk,D.M., and D.T.Tingey. 1982. Phytotoxicity of air pollutants: Evidence for phytodetoxification of SO_2 but not O_3. Plant Physiology 74: 399-1105.

Pinkerton,J.E., and A.S.Lefond. 1987. The characterization of ozone data for sites located in forested areas of the eastern United States. Journal of Air Pollution Control Association 37: 1005-1010.

Pye,J.M. 1988. Impact of Ozone on the Growth and Yield of Trees: A Review. Journal of Environmental Quality 17,3: 347-360.

Reich,P.B. 1987. Quantifying plant response to ozone: A unifying Theory. Tree Physiology 3: 63-91.

Sasek,T.W., and C.J.Richardson. 1989. Effects of chronic doses of ozone on loblolly pine: Photosynthetic characteristics in the third growing season. Forest Science 35,3: 745-755.

SAS Institute Inc. 1982. SAS user's guide. SAS Institute Inc., Cary, NC. p. 119-210.

Sheffield,R.M., N.D.Cost, W.A.Bechtold, and J.P.McClure. 1985. Pine growth reductions in the southeast. USDA Forest Service Recourse Bulletin SE-83.

Snedecor,G.W. and W.G.Cochran. 1967. Statistical Methods. Sixth edition. Iowa State University Press. Ames, Iowa. P. 237-239.

Tingey,D.T., and W.E.Hogsett. 1985. Water Stress Reduces Ozone Via a Stomatal Mechanism. Plant Physiology 77: 944-947.

Wiselogel,A.E., J.K.Bailey, R.J.Newton, and F.Fong. 1991. Growth response of loblolly pine seedlings to ozone fumigation. Environmental Pollution 70,3. (In Press).

Zobel,B.J., and J.T.Talber. 1984. Applied Forest Tree Improvement. John Wiley and Sons, NY, NY. 255 p.

GENERAL SESSION I

INTEGRATION OF BIOTECHNOLOGY
INTO APPLIED RESEARCH

THE USE OF MOLECULAR MARKERS TO DETECT HYBRIDIZATION IN INTROGRESSION ZONES

Y.A. El-Kassaby [1,2] and J.E. Carlson [2,3]

Abstract.--Operational spruce [Sitka (*Picea sitchensis*), white (*P.glauca*), and Engelmann (*P. engelmannii*)] seedlots collected from suspected zones of introgression often contain pure, mixed or hybrid seed of these three species. The contrasting seedling growing cultural requirements between Sitka and interior (white and Engelmann) spruce pose a seedling production problem and seedling quality is substantially affected. On the other hand, hybrids or mixes between interior spruce do not cause any operational problems due to their similar cultural requirements. The successful use of molecular markers [chloroplast (cpDNA) and mitochondrial DNA (mtDNA)] is illustrated and a comparison between molecular classification and operationally grown seedlots is presented. The production of species-specific unique mtDNA and cpDNA probes are used to determine their maternal and paternal inheritance, respectively. Finally, a protocol for molecular karyotyping is presented as an aid to determine the degree of hybridity at the nuclear genome level in parent trees of the interior spruce breeding program to separate interspecific superiority from that of intraspecific superiority.

Keywords: *Picea sitchensis, P. glauca, P. engelmannii*, cpDNA and mtDNA RFLPs, paternal and maternal inheritance, introgression.

INTRODUCTION

A total of 115 million spruce seedlings (Sitka [*Picea sitchensis* (Bong.) Carr.], white [*P. glauca* (Moench) Voss], Engelmann [*P. engelmannii* (Perry)], and their hybrids) are being produced annually in British Columbia nurseries for reforestation programs (Table 1). Engelmann and white spruce and their hybrid (spruce hybrids; Sx_i), collectively called "interior spruce" (Kiss 1976), alone reached an average planting of 105 million seedlings over the past four years (Table 1).

---

[1] Canadian Pacific Forest Products Limited, Tahsis Pacific Region, Saanich Forestry Centre, Saanichton, B.C. V0S 1M0 Canada. [2] Faculty of Forestry, University of British Columbia, Vancouver, B.C. V6T 1W5 Canada, [3] Biotechnology Laboratory, University of British Columbia, Vancouver, B.C. V6T 1W5 Canada.

Table 1. Number of spruce seedlings requested by sowing year (1988-1991) (in thousands).[1]

Species	1988	1989	1990	1991	Average
White spruce (Sw)	30,510	35,705	33,199	18,766	29,545
Engelmann spruce (Se)	39,218	39,830	35,364	31,622	36,509
Sitka spruce (Ss)	2,727	2,364	1,912	1,657	2,165
Sitka hybrids (Sx$_c$)[2]	3,587	5,811	3,629	3,166	4,048
Spruce hybrids (Sx$_i$)[3]	39,544	55,967	37,496	33,418	41,606
Total	115,586	139,677	111,600	88,629	455,492
Sitka hybrids (%)	3.1	4.2	3.3	3.6	3.6

[1] Personal communication (Mr. M. Pelchat, British Columbia Ministry of Forests, Silviculture Branch, Victoria, B.C., May 1991).

[2] Sitka hybrids; hybrids between Sitka spruce and any of the interior spruces (S$_i$) (white or Engelmann).

[3] Spruce hybrids; hybrids between white and Engelmann spruces.

The natural ranges of the Sitka, white, and Engelmann spruce overlap and several sympatric populations are present (Roche 1969; Krajina *et al.* 1982) (Fig. 1). The presence of these species in sympatric populations and the apparent lack of reproductive barriers to hybridization have led to the creation of several introgression zones (Little 1953; Daubenmire 1968; Roche 1969). Reforestation with spruce in the areas of Sitka by "interior spruce" hybridization is common, and operational natural stand seed collections provide the majority of seed required for seedling production.

Due to the typically sporadic nature of cone production in the wild, the cone bearing habit of the species (*i.e.,* cones being produced on the upper crown), and the narrow biological window for cone collection (i.e., time between cone maturation and seed shed), operational cone collections are being carried out by helicopters. The use of aerial cone-rakes to strip the cone crop from crop trees and their deposit on collection sites represents the major cone collection method practiced. In introgression zones, cone collections and subsequent seed crops may represent one species, a mixture of two or more species, or hybrids with various degrees of introgression.

Nursery cultural regimes required for the production of acceptable planting stock of Sitka and "interior spruce" in container nurseries are well documented (Brix 1972; Arnott 1974, 1979). Attempts to grow the two species in a common environment results in unacceptable stock quality. "Interior spruce" seedlings grown under the Sitka spruce cultural regime (*i.e.*, without extended photoperiod) will terminate their leader growth
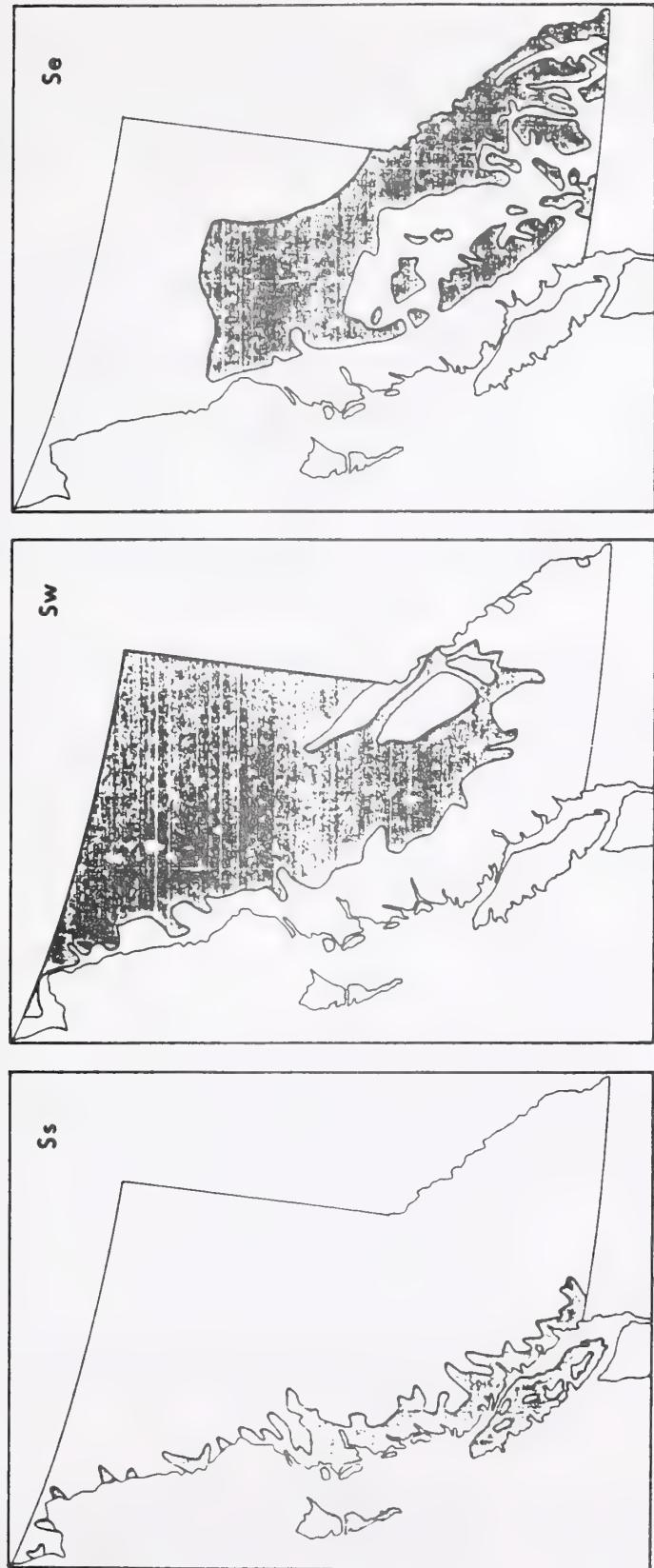
Figure 1. Map of British Columbia showing the natural range of Sitka spruce (Ss), white spruce (Sw), and Engelmann spruce (Se) (source; Krajina *et al.* 1982).

(*i.e.*, set bud) early, before reaching target height. Conversely, Sitka spruce grown under extended photoperiod produces unacceptably tall stock. Thus, the production of acceptable planting stock requires different light regimes for each species. Seed collected from introgression zones grown under any of the previously-mentioned light regimes produce stock of variable quality.

The distinction between "interior spruce" and their hybrid (Sx_i) has no operational significance since both species and their hybrid can be successfully grown under the same cultural regime and are planted on similar sites. Sitka hybrids (Sx_c) (*i.e.*, hybrids between Sitka and either white or Engelmann spruce) or Sitka/interior spruce mixes pose an operational seedling production problem; although they only represent 4% of the total spruce produced annually, a total of 3-6 million seedlings are still needed for reforestation (Table 1). The development of a reliable and cost-effective means of screening these seedlots would allow hybrids or mixed seedlots to be grown under appropriate cultural conditions for the predominant species resulting in saving most of the loss experienced during the seedling production phase.

This paper describes the use of species-specific molecular cytoplasmic organelle markers as a species classification method and its evaluation on an operational level. In addition, a protocol for molecular karyotyping is presented as an aid to determine the degree of hybridity at the nuclear genome level.

PROJECT SUMMARIES

Chloroplast DNA (cpDNA)

This section describes the methods used to identify Sitka, white, and Engelmann spruce and their hybrids/mixes using cpDNA. The extraction protocol of cpDNA from conifers (White 1986) and the first demonstration of cpDNA paternal inheritance in Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] (Neale *et al.* 1986) were instrumental to the development and the conceptual framework for this method. El-Kassaby *et al.* (1988) and Szmidt *et al.* (1988) presented the first evidence of species-specific cpDNA restriction patterns that were successful in identifying Sitka, white and Engelmann spruce from each other. Three restriction enzymes (*Bam*-HI, *Bcl*-I, and *Xba*-I) were used (Fig. 2). *Bam*-HI differentiated Sitka from "interior spruce," *Bcl*-I differentiated white from Engelmann spruce, and *Xba*-I confirmed results obtained from *Bam*-HI and *Bcl*-I (Fig. 2).

The identification of seedlots (pure or mixed) was based on three assumptions: 1) the mode of inheritance of cpDNA is paternal in spruce; 2) pure seedlots (*i.e.*, Sitka, white, and Engelmann) are identified with certainty (i.e., should show banding patterns identical to reference species), and 3) mixed/hybrid seedlots should show a mixture of
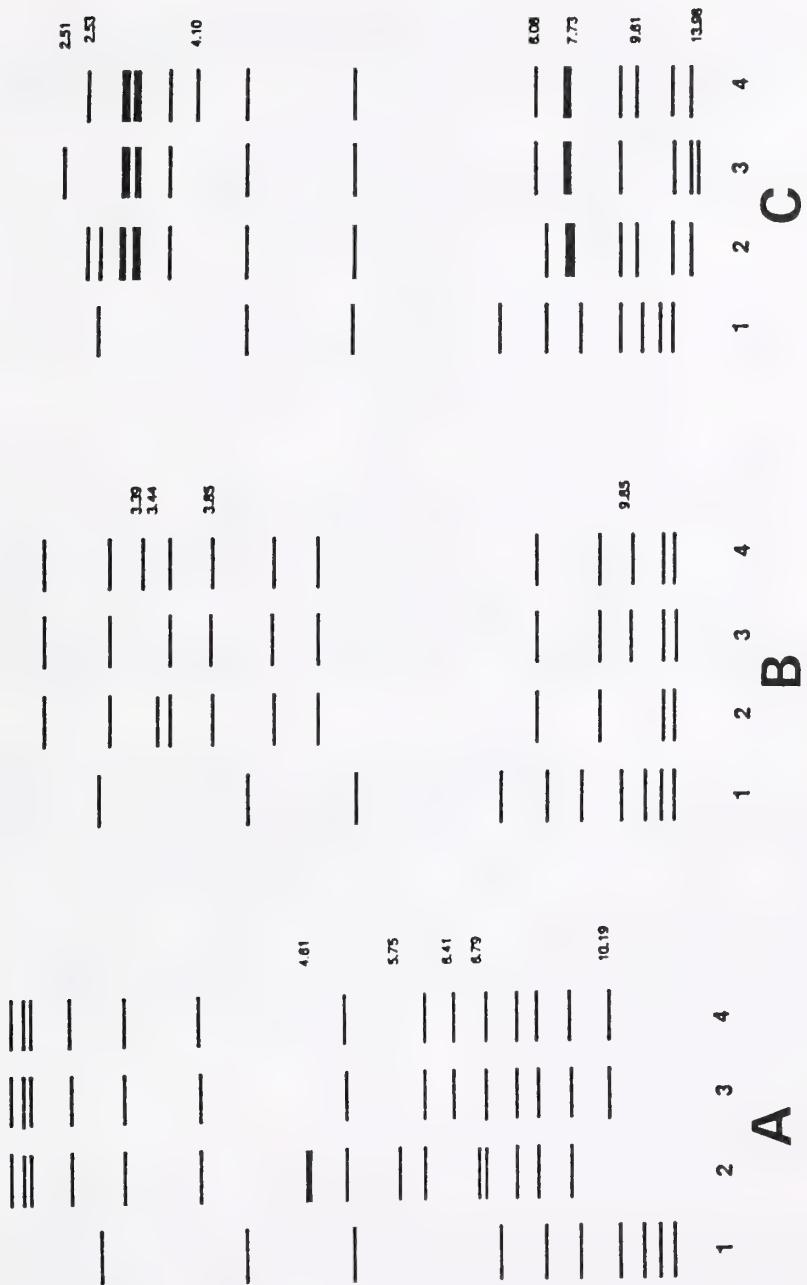
Figure 2. Schematic drawing illustrating cpDNA restriction patterns from three spruce species generated by: (A) *Bam*-HI, (B) *Bcl*-I, and (C) *Xba*-I. Lane 1: 1kb ladder (BRL); Lane 2: Sitka spruce; Land 3: white spruce; Lane 4: Engelmann spruce (source; El-Kassaby *et al.* 1988).

43

restriction fragments that are unique to each species concurrently, due to the presence of more than one species' pollen in the introgression zone. The concurrent appearance of two or more species' unique restriction fragments is expected when cpDNA is extracted from a bulk sample of needles obtained from 40-60 seedlings that originated from a seedlot collected from introgression zones.

A paternal mode of inheritance of cpDNA in conifers has been demonstrated for several species (Neale *et al.* 1986, 1989; Szmidt *et al.* 1987, 1988; Wagner *et al.* 1987, 1989; Neale and Swederoff 1989; Stine *et al.* 1989; Stine and Keathley 1991; White 1990; Sutton *et al.* 1991b), however, occasional biparental inheritance was detected in some cases (Szmidt *et al.* 1987; Govindaraju *et al.* 1988; White 1991; Sutton *et al.* 1991a). Although cpDNA is regarded as highly conservative (Palmer 1987), intra-specific variation has been reported for several species (Wagner *et al.* 1987; Govindaraju *et al.* 1989; White 1990; Ali *et al.* 1991). However, the chance of observing all unique fragments collectively as intrapopulational variation in one sample was considered to be highly unlikely (El-Kassaby *et al.* 1988; Szmidt *et al.* 1988).

This approach was tested by Szmidt *et al.* (1988) on five seedlots and an operational scale screening was carried out by El-Kassaby *et al.* (1988) on 20 seedlots. Although this method was effective in correctly classifying unknown seedlots (Table 2), it required the tedious isolation of purified cpDNA. A simplified approach, based on a rapid Southern plot hybridization protocol with a species-specific DNA probe that yielded quantitative determination of one species in a mixed seedlot was developed by Sutton *et al.* (1991a).

Detailed descriptions of identification and cloning of chloroplast probes for distinguishing Sitka and interior spruce are documented in Sutton *et al.* (1991a). Briefly, *Bam*-HI digested cpDNA from mature individuals revealed a 10.5 kb (kilobases) fragment unique to white spruce and fragments of 4.3 and 5.5 kb unique to Sitka spruce. Probes were selected based on their ability to distinguish each species, as well as the lack of hybridization with the nuclear fractions. Two probes (pSS4 and pSS6) were selected and results showed that either one could be used. The smaller probe (pSS4) was used in the routine screening.

cpDNA Seedlot Classification

Needle samples (0.5 g) from 200, two-week-old germinants were pooled and total DNA was isolated for 13 operational seedlots (Fig. 3a). When mixed/hybrid seedlots were identified, the hybridizing bands were scanned using a densitometer with area integration to determine the percent contribution of the two species (see Sutton *et al.* 1991a for details). Density is compared to a standard curve that was generated from a series of mixtures of white and Sitka spruce total DNA standards (Fig. 4). The detection limit of the procedure allows less than 5% of either Sitka or interior spruce to be
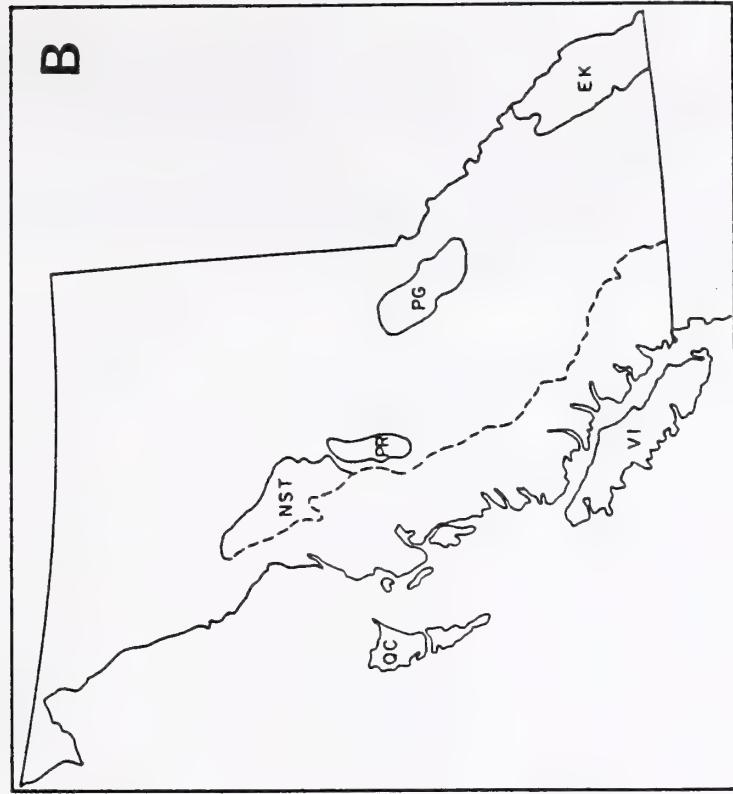
Figure 3. (A) Map of British Columbia showing the locations of the 13 spruce seedlots classified. (B) Map of British Columbia showing the locations of trees used in the inheritance study and tested trees (EK, East Kootenay; PG, Prince George; VI, Vancouver Island; QC, Queen Charlotte Islands; PR, Prince Rupert, Smithers selection unit; NST, Nass Skeena Transition) (source; El-Kassaby *et al.* 1988, Sutton *et al.* 1991a and b).

detected [Engelmann spruce quantification yielded similar results to white spruce (Sutton *et al.* 1991a)]. In addition, the classification of these 13 seedlots based on diagnostic probes on total DNA is compared to the restriction fragment banding pattern of purified cpDNA along with their nursery performance (Table 2). Generally, both methods gave similar results, however, the use of diagnostic probes eliminated the tedious work required for isolating pure cpDNA samples and allowed the quantitative determination of each species in the mix.
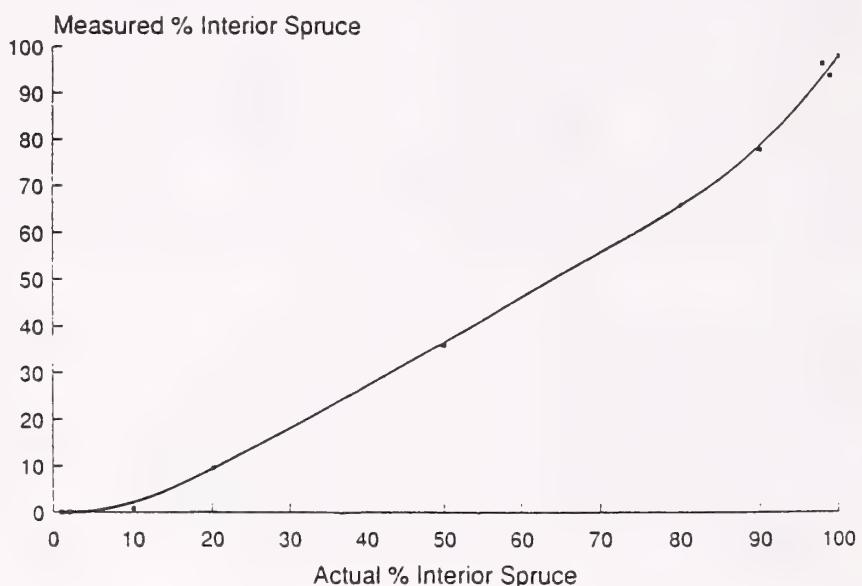
Figure 4. Standard curve for determination of cpDNA content of interior and Sitka spruce (source; Sutton *et al.*, 1991a).

Mitochondrial DNA (mtDNA)

The previous section demonstrated the utility of cpDNA genome as a pollen donor marker in interspecific mating events. If the mtDNA genome is maternally inherited in spruce, then a combination of probes that are capable of distinguishing both the maternal (*i.e.*, mtDNA) and the paternal (*i.e.*, cpDNA) parents of an individual would be of great use as a first step in determining the pedigree of that individual.

The mode of inheritance of mtDNA in conifers has been studied for several species representing three families. Loblolly pine (*Pinus taeda* L.) (Neale and Sederoff 1989), spruce (Sitka, white and Engelmann) (Sutton *et al.* 1991b), and Douglas-fir (D. B. Neale, personal communication, May 1991) of the Pinaceae exhibited maternal inheritance while coastal redwood (*Sequoia sempervirens* D. Don Endl.) of the Taxodiaceae (Neale *et al.* 1989) and incense-cedar [*Calocedrus decurrens* (Torr.) Florin] of the Cupressaceae (Neale *et al.* 1991) showed paternal inheritance.

Table 2. Comparison between methods used to classify spruce seedlots based on cpDNA analyses and nursery trial.

Seedlot Registration		Classification		
		El-Kassaby[1]	Sutton[2]	Woods[3]
1855	Sx_c	Sw	Sx_c (40)	Ss†
2647	Ss	Ss	Sx_c (<5)	Ss
2762	Sx_c	Ss/Sw/Se	Sx_c (50)	Ss†
2793	Sx_c	Ss	Ss (--)	Ss
2856	Sx_c	Ss	Ss (--)	Ss
7754	Sx_i	Ss	Ss (--)	Ss
7755	Sx_i	Ss	Ss (--)	Ss
7757	Sx_i	Ss	Sx_c (<5)	Ss
7761	Sx_i	Ss	Ss (--)	Ss
8602	Se	Sw/Se	S_i (100)	S_i
9963	Se	Ss	Ss (--)	Ss
9964	Se	Ss	Sx_c (<5)	Ss
29201	Sx_i	Ss/Sw/Se	Sx_c (60)	Ss†

[1] El-Kassaby *et al.* (1988); classification of spruce seedlots based on purified cpDNA.

[2] Sutton *et al.* (1991a); classification of spruce seedlots based on hybridization to DNA probe. Numbers in parentheses represent the percentage of interior spruce as determined by densitometry analysis.

[3] Woods (1988); recommended growing regime based on nursery comparison of height, caliper, root and shoot dry weights, and days to bud set.

† Seedlots classified as mix/hybrid.

The methods of isolating mtDNA and the preparation of probes that are capable of distinguishing among the three spruce species (Sitka, white and Engelmann) are described in Sutton *et al.* (1991b). In their study, a polymorphism which distinguished white spruce from both Sitka and Engelmann spruce was found by using *Hpa*-I digestion and probing with pWSm1. In this case, a 15 kb fragment was found to be unique to white spruce while a 20kb fragment was unique to both Sitka and Engelmann spruce. Additionally, hybridization with pWSm2 probe in combination with *Sma*-I digestion provided unique polymorphisms that can distinguish all three species as follows: Engelmann spruce contained a 23 kb band, white spruce was characterized by a 8.1 kb band, and Sitka spruce individuals were of two types, 21 kb or 31 kb. The use of these probes in the identification of spruce individuals is presented below.

Paternal Inheritance of cpDNA and Maternal Inheritance of mtDNA in Spruce

Sutton *et al.* (1991b) conducted some reciprocal interspecific crosses demonstrating the mode of inheritance of both cpDNA and mtDNA using the previously described probes. In their study, all investigated progenies conformed to expectations, providing further support for previous observations on Pinaceae. Sutton *et al.* conducted crosses between individuals that were selected to represent a "typical" species. White spruce trees were obtained from low-elevation areas in the Prince George region, Engelmann spruce from high-elevation areas in the East Kootenay region, and Sitka spruce from low-elevation areas of Vancouver Island and the Queen Charlotte Islands (Fig. 3b). A total of 27 progenies were investigated for cpDNA while 32 were studied for mtDNA (Sutton *et al.* 1991b) (Table 3).

Table 3. Results of cpDNA and mtDNA inheritance in some reciprocal interspecific crosses (source; Sutton *et al.* 1991b).

Cross (female x male)[1]	Probe[2]	Organelle	Inheritance	Sample Size
Ss x Se	pSS4	cp	paternal	6
	pWSm2	mt	maternal	6
Se x Ss	pSS4	cp	paternal	7
	pWSm2	mt	maternal	5
Ss x Sw	pSS4	cp	paternal	7
	pWSm1	mt	maternal	7
Sw x Ss	pSS4	cp	paternal	7
	pWSm1	mt	maternal	7
Se x Sw	pWSm2	mt	maternal	7

[1] See Table 1 for explanation.
[2] See Sutton *et al.* 1991a and b for description.

Identification of Parentage of Trees from Introgression Zones

Sutton *et al.* (1991b) used both the mitochondrial and chloroplast probes to determine the parentage of 14 individual trees selected from Prince Rupert region (PR) (Fig. 3b). This region is located east of the recognized Nass Skeena Transition (NST) introgression zone (Roche 1969). It is noteworthy to mention that this area is in the eastern watershed of the Coast Mountains where interior spruce is expected to be the dominant species. The maternal and parental lineage of these individuals are summarized in Table 4. Only one individual (#86) produced unique bands of interior spruce for both cpDNA and mtDNA (Table 4). Of the remaining 13 individuals, all but one (#14) produced a cpDNA banding pattern that is typical of interior spruce

(Table 4). This was due to the high frequency of interior spruce pollen present in this region's pollen pool. However, the mtDNA banding pattern of these 13 individuals was dominated by the Sitka spruce type (12 out of 13) and only one tree produced a pattern that is similar to Engelmann spruce (#14). These results demonstrate that the introgression zone is larger than expected and that Sitka spruce is being maintained mainly as a seed donor.

Table 4. Parentage identification for 14 individual trees selected from the Prince Rupert, B.C. region (source; Sutton et al. 1991b).

Tested Tree Number	Unique Bands (in kb)[1]		Species Designation[2]	
	probe mtDNA pWSm2	probe cpDNA pSS4	mt	cp
1	21	10.5	Ss	S_i
2	21	10.5	Ss	S_i
4	21	10.5	Ss	S_i
5	21	10.5	Ss	S_i
14	23	4.3	Se	Ss
19	21	10.5	Ss	S_i
20	21	10.5	Ss	S_i
33	21	10.5	Ss	S_i
75	21	10.5	Ss	S_i
78	21	10.5	Ss	S_i
85	21	10.5	Ss	S_i
86	23	10.5	Se	S_i
109	21	10.5	Ss	S_i
110	21	10.5	Ss	S_i

[1] From Sutton et al. (1991a and b).
[2] See Table 1 for explanation.

Limitations of Parentage Identification Based on Cytoplasmic Genomes

While cpDNA and mtDNA analyses are valid first steps in evaluating seedlots or individuals, they are limited to identifying only the cytoplasmic genomes and immediate pollen/seed parents (El-Kassaby et al. 1988; Szmidt et al. 1988; Sigurgeirsson et al. 1990, 1991; Stine and Keathley 1990; Sutton et al. 1991a and b). CpDNA and mtDNA patterns will not reveal if the parents were hybrids or if they were pure species. Incorrect classification could occur if both parents were hybrids and true hybrid progeny will go undetected. This situation is illustrated in the following hypothetical cross:

Female	x	Male	P_1
mtDNA (Sw)		mtDNA (Ss)	
cpDNA (Ss)		cpDNA (Sw)	
↓		↓	
mtDNA (Sw)		cpDNA (Sw)	

<div align="center">

mtDNA (Sw)

cpDNA (Sw) F_1

</div>
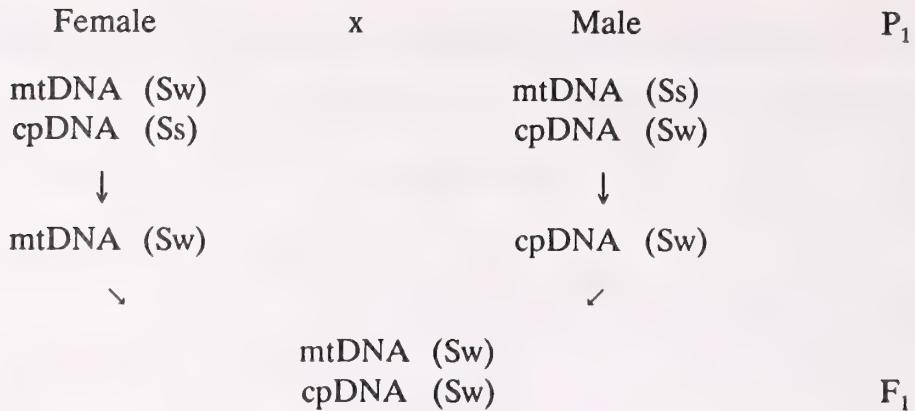
In this example, the progeny is classified as a "pure" white spruce based on the cytoplasmic genomes, however, in reality it is a cross of two hybrids and at the nuclear level is hybrid in nature.

At present, the majority of spruce seed used in reforestation are from natural stand collections. This situation will eventually change when seed orchards reach production phase. These seed orchards will also undergo genetic upgrading (*i.e.*, roguing) based on progeny test information. Some of the progeny test families could have been the product of interspecific crosses and might exhibit phenotypic superiority due to hybrid vigour and not their actual intraspecific variation. Therefore, the determination of the exact degree of introgression in the breeding population is of importance and the DNA content at the nuclear genome level is warranted.

Traditionally, following introgression, the detection of chromosomes or chromosome segments from one species into another or one breeding population into another has relied upon cytogenetics. When detailed karyotypes are available, individual chromosomes can be identified and the movement of large blocks of chromosomal material can be mapped using various cytological staining techniques. However, no satisfactory method for karyotyping members of the genus *Picea* are available. We have been able to use methods developed by Kiss (1973) to visualize all of the spruce chromosomes in root tip squashes. However, no cytological staining technique provides detailed or unambiguous chromosome identification in *Picea* spp.

However, molecular cytogenetics provides a powerful approach to the identification and detailing of chromosome structure. The field of molecular cytogenetics has developed around the technique of *in situ* hybridization. *In situ* hybridization is a physical genome mapping technique developed by Gall and Pardue (1969) in which labelled DNA fragments are hybridized to cytological preparations of chromosomes. Individual DNAs are mapped to specific chromosome regions, usually heterochromatic regions, to obtain a hybridization pattern that is species specific. We are in the process of developing such a molecular karyotype for the Engelmann, Sitka, and white spruce species. With this molecular karyotype information, we will be able to

determine the origin of each chromosome in natural spruce hybrids. We also anticipate being able to follow the introgression of much smaller chromosome segments than can be followed with cytological staining techniques.

Our physical mapping capability derives from the more recent development of the Fluorescence *In Situ* Hybridization (FISH) technique and the use of confocal microscopy. With FISH (Lichter *et al.* 1990) and confocal imaging (Albertson 1991), resolution can be obtained at the 10 Mbp range for metaphase chromosomes and the 50 to 1000 kbp range for interphase nuclei (Trask *et al.* 1991). With FISH, the DNA probe is labelled with biotin either directly or by DNA polymerase incorporation. The hybridization signal is detected either with an anti-biotin fluorochrome conjugated antibody or fluorochrome conjugated streptavidin, followed by signal amplification if necessary (Fig. 5). Confocal microscopy in particular has an advantage for *in situ* hybridization with conifer root tips which yield thick squashes making it often difficult to observe all chromosomes in a spread. With the BioRAD MRC-600 confocal microscope, individual optical sections are taken through the preparation in each focal plane. All of the chromosomes are then overlaid in one image. The BioRad MRC-600 confocal microscope image analysis software also includes cut and paste routines for aligning the chromosomes and measurement routines for quantifying banding patterns along each chromosome. With this capability, we can locate and identify the source of individual chromosome or chromosome segments in a suspected hybrid individual or seedlot.

ACKNOWLEDGEMENTS

Figure 5. Protocol for fluorescence *in situ* hybridization (FISH) to conifer root tip chromosomes (source; Brown, G.R., and J.E. Carlson, unpublished).

LITERATURE CITED

Albertson, D. 1991. Non-isotopic *in situ* hybridization. BioRad Laboratories. Application Note: 02.

Ali, I.F., D.B. Neale, and K.A. Marshall. 1991. Chloroplast DNA restriction fragment length polymorphism in *Sequoia sempervirens* D. Don Endl., *Pseudotsuga menziesii* (Mirb.) Franco, *Calocedrus decurrens* (Torr.), and *Pinus taeda* L. Theor. Appl. Genet. 81:83-89.

Arnott, J.T. 1974. Growth response of white and Engelmann spruce provenances to extended photoperiod using continuous and intermittent light. Can. J. For. Res. 14:69-75.

Arnott, J.T. 1979. Effect of light intensity during extended photoperiod on growth of amabilis fir, mountain hemlock, and white and engelmann spruce seedlings. Can. J. For. Res. 19:82-89.

Brix, H. 1972. Growth response of Sitka spruce and white spruce seedlings to temperature and light intensity. Can. For. Serv., Pac. For. Res. Cent., Info. Rep. BC-X-74.

Doubenmire, R. 1968. Some geographic variations in *Picea sitchensis* and their ecological interpretation. Can. J. Bot. 46:787-798.

El-Kassaby, Y.A., A. Sigurgeirsson, A.E. Szmidt. 1988. The use of restriction analysis of chloroplast DNA in classifying hybrid spruce seedlots. P. 67-88 *in* Molecular Genetics of Forest Trees, Proc. of the Frans Kempe Symposium, Umeå, Sweden, J.-E. Hällgren (ed.).

Gall, J.G. and M.L. Pardue. 1969. Formation and detection of RNA-DNA hybrid molecultes in cytological preparations. Proc. Natl. Acad. Sci. (USA) 63:378-382.

Govindaraju, D.R., B.P. Dancik, and D.B. Wagner. 1989. Novel chloroplast DNA polymorphism in a sympatric region of two pines. J. Evol. Biol. 2:49-59.

Kiss, G. 1973. Preliminary karyotype analyses in *Picea*. British Columbia Ministry of Forests Report E.P. 670.98.

Kiss, G. 1976. Plus-tree selection in British Columbia. P. 24-31 *in* Proc. 15th Can. Tree Imp. Assoc., Part 1, E.K. Morgenstern (ed.).

Krajina. V.J., K. Klinka, and J. Worrall. 1982. Distribution and ecological characteristics of trees and shrubs of British Columbia. J&M Publishing, Vancouver, B.C.

Lichter, P., C.C. Tang, K. Call, G. Hermanson, G.A. Evans, D. Housman and D.C. Ward. 1990. High resolution mapping of human chromosome 11 by *in situ* hybridization with cosmid clones. Science 247:64-69.

Little, E.L. 1953. A natural hybrid species in Alaska. J. For. 41:745-746.

Neale, D.B., N.C. Wheeler, and R.W. Allard. 1986. Paternal inheritance of chloroplast DNA in Douglas-fir. Can. J. For. Res. 16:1152-1154.

Neale, D.B. and R.R. Sederoff. 1989. Paternal inheritance of chloroplast DNA and maternal inheritance of mitochondrial DNA in loblolly pine. Theor. Appl. Genet. 77:212-216.

Neale, D.B., K.A. Marshall, And R.R. Sederoff. 1989. Chloroplast and mitochondrial DNA are paternally inherited in *Sequoia sempervirens* D. Don. Endl. Proc. Natl. Acad. Sci. (USA) 86:9347-9349.

Neale, D.B., K.A. Marshall, and D.E. Harry. 1991. Inheritance of chloroplast and mitochondrial DNA in incense-cedar [*Calocedrus decurrens* (Torr.) Florin]. Can. J. For. Res. (in press).

Palmer, J.D. 1987. Chloroplast DNA evolution and biosystematic uses of chloroplast DNA variation. Am. Nat. 130:S6-S29.

Roche, L. 1969. Introgressive hybridization in the spruce species of British Columbia. P. 249-270 *in* Proc. 11th Comm. For. Tree Breeding in Canada, C.W. Yeatman (ed.).

Sigurgeirsson, A., A.E. Szmidt, and J.N. Alden. 1990. A molecular study of interspecific hybridization in the spruce complex of Alaska. P. 6.14-6.23 *in* Joint Meeting of Western For. Genet. Assoc. and IUFRO Work. Parties - S2-02-05, 06, 12 and 14, Olympia, Washington.

Sigurgeirsson, A., A.E. Szmidt, and B. Korpinska. 1991. Alaskan *Picea sitchensis* populations infiltrated with *Picea glauca* genes: A study using DNA markers. P. 197-207 *in* Biochemical markers in the population genetics of forest trees, Fineschi, S., M.E. Malvolti, F. Cannata, and H.H. Mattemer (eds). SPB Academic Publishing bv, The Haugue, The Netherlands.

Stine, M., B.B. Sears, and D.E. Keathley. 1989. Inheritance of plastids in interspecific hybrids of blue spruce and white spruce. Theor. Appl. Genet. 78:768-774.

Stine, M., and D.E. Keathly. 1990. Paternal inheritance of plastids in Engelmann spruce x blue spruce hybrids. J. Hered. 81:443-446.

Sutton, B.C.S., D.J. Flanagan, and Y.A. El-Kassaby. 1991a. A simple and rapid method for estimating representation of species in spruce seedlots using chloroplast DNA restriction fragment length polymorphism. Silvae Genet. (in press).

Sutton, B.C.S., D.J. Flanagan, J.R. Gawley, C.H. Newton, D.T. Lester, and Y.A. El-Kassaby. 1991b. Inheritance of chloroplast and mitochondrial DNA in *Picea* and composition of hybrids from introgression zones. Theor. Appl. Genet. (in press).

Szmidt, A.E., T. Alden, and J.-E. Hällgren. 1987. Paternal inheritance of chloroplast DNA in *Larix*. Plant Mol. Biol. 9:59-64.

Szmidt, A.E., Y.A. El-Kassaby, A. Sigurgeirsson, T. Alden, D. Lindgren, and J.-E. Hällgren. 1988. Classifying seedlots of *Picea sitchensis* and *P. glauca* in

zones of introgression, using restriction analysis of chloroplast DNA. Theor. Appl. Genet. 76:841-845.

Trask, B., A. Fertitta, M. Christensen, B. Branndiff, L. Gordon, A. Copeland, H. Massa, K. Tynan and A. Carrano. 1991. Two-color fluorescence *in situ* hybridization mapping of chromosome 19. Cold Spring Harbor Laboratory Conference in Genome Mapping and Sequencing, Cold Spring Harbor, New York. (Abstracts). P. 209.

Wagner, D.B., G.R. Furnier, M.A. Saghai-Maroof, S.M. Williams, B.P. Dancik, and R.W. Allard. 1987. Chloroplast DNA polymorphisms in lodgepole and jack pine and their hybrids. Proc. Natl. Acad. Sci. (USA) 84:2097-2100.

Wagner, D.B., D.R. Govindaraju, C.W. Yeatman, and J.A. Pitel. 1989. Paternal chloroplast DNA inheritance in a diallel cross of jack pine (*Pinus banksiana* Lamb.). J. Hered. 80:483-485.

White, E.E. 1986. A method for extraction of chloroplast DNA from conifers. Plant. Mol. Biol. Rep. 4:98-101.

White, E.E. 1990. Chloroplast DNA in Pinus monticola. 2. Survey of within-species variability and detection of heteroplasmic individuals. Theor. Appl. Genet. 74:251-255.

Woods, J.H. 1988. Sitka-interior hybrid spruce seedlot trials. Unpublished Report. B.C. For. Serv., Res. Branch.

MASS PROPAGATION OF SOMATIC EMBRYO-DERIVED PLANTLETS OF YELLOW-POPLAR FOR FIELD TESTING

S.A. Merkle[1], S.E. Schlarbaum[2], R.A. Cox[3], and O.J. Schwarz[4]

Abstract. Somatic embryogenesis is generally regarded to be the most promising tissue culture regeneration system for mass propagation of desirable genotypes. However, few field tests employing somatic embryo-derived trees have been initiated, primarily due to inadequate numbers of somatic embryo-derived plantlets ("emblings"). In this study, we report the development of a system for large-scale production of yellow-poplar somatic embryos followed by high-frequency conversion, and its integration into a collaborative project to field test the tissue-cultured material. Embryogenic yellow-poplar suspension cultures were initiated from immature zygotic embryos resulting from controlled pollinations of trees growing in a University of Tennessee yellow-poplar breeding orchard near Knoxville, TN. Following testing of suspension cultured lines for their ability to produce somatic embryos and emblings, 9 lines were chosen for mass production. To produce synchronous populations of mature somatic embryos, proembryogenic masses (PEMs) of each line were size-fractionated and the desired fraction was cultured on filter paper overlayed on a plate of semisolid basal medium. Following 2 weeks of incubation under these conditions, mature somatic embryos were transferred to plates of a semisolid basal germination medium and shipped to cooperators at the University of Tennessee, Knoxville. Germinants were transferred to GA7 vessels containing plantlet development medium. Conversion rates averaged approximately 66 percent. Following up to 4 months in GA7 vessels, emblings were transferred to potting mix in planting containers and acclimatized in a misting greenhouse. Following 2 months of acclimatization, approximately 5500 emblings, representing 9 clones, were transferred to larger containers and placed in a shade house for further growth.

Keywords: *Liriodendron tulipifera*, somatic embryogenesis, conversion

---

[1]School of Forest Resources, University of Georgia, Athens, GA 30602, [2]Department of Forestry, Wildlife and Fisheries, University of Tennessee, Knoxville, TN 37901, [3]Tennessee Division of Forestry, Knoxville, TN 37901, and [4]Department of Botany, University of Tennessee, Knoxville, TN 37901

INTRODUCTION

Among the plant tissue culture techniques that have been applied to forest tree species, somatic embryogenesis, the asexual production of embryo-like structures, has received increasing attention in recent years. During this time, great progress has been reported in the initiation of embryogenic cultures of both coniferous and hardwood species (cf. reviews by Tulecke 1987, Wann 1989). Somatic embryogenesis is currently viewed as a tool with great potential to be applied for mass propagation of desirable genotypes of agronomic, horticultural and forest tree species. Compared to both conventional and other *in vitro* propagation systems, many embryogenic systems offer high multiplication rates, especially when they can be maintained as suspension cultures. Even more attractive economies of scale may be possible if bioreactor and continuous culture technologies can be applied to embryogenic systems (e.g. Styer 1987, Stuart et al. 1987). Furthermore, the products of these cultures are virtually complete propagules in themselves, requiring no separate shoot elongation or rooting steps to produce plantlets. This property of somatic embryos has opened the possiblity that they may be adapted for direct delivery to greenhouse or field as "artificial seeds" (e.g. Redenbaugh et al. 1986; Kitto and Janick 1985; Gupta and Durzan 1987). These features of embryogenic systems serve to lower labor inputs relative to those required for other *in vitro* propagation methods.

Somatic embryogenesis has taken on an added level of importance with the advent of plant gene transfer technology. Several of the features of embryogenic systems make them amenable to gene transfer via both *Agrobacterium*-Ti plasmid-mediated and direct gene transfer techniques. For example, the capacity for embryogenic cultures of walnut (*Juglans regia*) to undergo cycles of repetitive somatic embryogenesis has been employed to produce transformed embryos and plantlets of that species, following *Agrobacterium*-mediated gene transfer (McGranahan et al. 1989, 1990). Recently, embryogenic suspension cultures of such agronomic species as maize and cotton have been transformed using a direct gene transfer technique known as microprojectile bombardment, in which DNA is delivered to plant cells via metal particles which are shot into them with a "gun" (Gordon-Kamm et al. 1990, Finer and McMullin 1990). Transformed plantlets of yellow-poplar (*Liriodendron tulipifera*) have also been regenerated following microprojectile bombardment of embryogenic suspension cultures (Wilde, Meagher and Merkle, submitted).

Despite the great potential for embryogenic systems to be adapted for mass propagation and gene transfer for forest trees, there remain several major obstacles to be overcome before somatic embryogenesis can be integrated with operational tree breeding programs and begins to make a real contribution to applied forest tree improvement and/or reforestation. One major problem is that the majority of forest tree embryogenic systems have employed immature tissues as explants. For full advantage to be taken of propagation via this technique, systems using explants from mature, genetically-proven material will have to be developed. Until such systems are available, there is much information that can be gained by employing cultures derived from immature tissues, which are presently available. In addition, recent advances in cryopreservation of embryogenic cultures (e.g. Shillito et al. 1989) have made possible the long term storage of potential superior genotypes while somatic embryo-derived trees of the same clone are evaluated for field performance.

Importance of field testing

One important question that can be addressed using available embryogenic material concerns the potential for somatic embryo-derived plantlets ("emblings") to be used operationally as propagules. In order to judge the true usefulness of these trees, their *ex vitro* survival and growth must be evaluated in greenhouse, nursery and field situations. As seedlings are currently the primary propagules used in forestry, they are the standard to which emblings must be compared. An important part of this comparison will be determining if the per unit costs of emblings are competitive with those of seedlings. In addition, since one of the presumed advantages of utilizing tissue culture propagation systems is the production of clonal plant material, a major consideration is embling performance with regard to clonal fidelity. Finally, as the reality of genetically-engineered trees draws nearer, information on field performance of somatic embryo-derived trees will be needed to establish a baseline to which to compare the genetically-engineered trees when they are tested in the field.

Previous field studies of tissue culture-derived trees

Given the great progress with embryogenic systems for forest trees and the importance of information concerning the *ex vitro* performance of the trees, one might expect that a number of such tests would be underway to answer these questions. On the contrary, information on the field performance of forest tree emblings is currently unavailable. To date, the only report of a large-scale, *ex vitro* evaluation of somatic embryo-derived trees in North America has been that of Webster et al. (1990), in which 1200 emblings of of interior spruce (*Picea glauca*, *Picea engelmannii*, and natural hybrids) were tested for nursery performance. They found that growth rates, final height, shoot and root morphology, and frost hardiness were similar for emblings and seedlings following the first growing season. Probably the woody perennial for which *ex vitro* performance of emblings has been most extensively tested is the oil palm (*Elaeis guineensis*). Field trials established by Unilever involved approximately 30,000 plantlets, representing at least 50 clones. (Choo 1990). Early results indicated that plantlets within a clone were highly uniform, especially for highly heritable characters (Corley et al. 1981).

Reports on field tests of forest trees derived from other tissue culture regeneration systems (micropropagation, organogenesis) indicate that plantlets derived via these techniques perform similarly to seedlings, although some characteristics of plantlets may differ from seedlings of the same age. Micropropagated plantlets of hardwoods including *Amelanchier*, *Betula*, *Populus*, *Quercus*, *Ulmus*, *Salix* and *Sorbus* have generally performed very well in the field (Ahuja 1987; Chalupa 1987; McCown and McCown 1987). Tissue culture derived plantlets of teak (*Tectona grandis*) actually outperformed seedling-derived plants when growth increments were compared for the first three years of a field test (Mascarenhas et al. 1987). However, special precautions may be necessary to prevent root circling and deformation in micropropagated hardwood transplants (McCown and McCown 1987). For example, sweetgum (*Liquidambar styraciflua*) plantlets derived from adventitious shoots and planted in a raised nursery bed suffered from poor root form which limited their usefulness for field establishment (Sommer et al. 1985).

Among coniferous forest species, large numbers of micropropagated trees of such species as redwood (*Sequoia sempervirens*) and radiata pine (*Pinus radiata*) are being produced for field planting (Boulay 1990; Thorpe et al. 1991). In the United States, the North Carolina State University Project on Tissue Culture established 16 field plantings of loblolly pine (*Pinus taeda*) plantlets derived from adventitious buds (Amerson et al. 1988). In these tests, loblolly pine plantlets were significantly smaller than seedlings after 1 and 2 years of growth, suggesting that the plantlets experienced an adaptation period to field conditions. However, by the fourth year, growth increments were similar for the plantlets and seedlings. For unknown reasons, plantlets displayed lower rates for fusiform rust infection than seedlings, and their morphology was more mature than similarly aged trees of seedling origin. Douglas-fir (*Pseudotsuga menziesii*) plantlets displayed a similar lag in height growth compared to seedlings, although annual growth increments were similar (Ritchie and Long 1986).

Although lack of resources may have prevented initiation of field tests of somatic embryo-derived trees in some cases, it is likely that the primary factor limiting the establishment of such tests is that most forest tree embryogenic systems reported to date are simply not capable of high frequency production of emblings exhibiting *ex vitro* survival and growth. For example, in one of the few reports to provide quantitative data on conversion of forest tree somatic embryos to plantlets, Becwar et al. (1989) calculated the rate of overall efficiency of plantlet recovery from a Norway spruce (*Picea abies*) embryogenic system to be only 0.5%. Although conversion rates for conifer somatic embryos continue to improve with new techniques (e.g. Roberts et al. 1990; Krogstrup 1990), most systems still must be vastly improved before large populations of clonal plantlets are available for testing. Here, we describe the development of an embryogenic regeneration system for the hardwood forest tree yellow-poplar (*Liriodendron tulipifera*) to a stage where thousands of somatic embryo-derived trees can be routinely produced for *ex vitro* establishment. Using this system, we have recently initiated a project that integrates tissue culture research and practical tree improvement to test somatic embryo-derived trees, derived from select germplasm, for early growth and survival during acclimatization, greenhouse, nursery and field phases.

INITIATION OF YELLOW-POPLAR EMBLING TESTS

Establishment of embryogenic yellow-poplar cultures

Collaboration between laboratories at the University of Georgia (UGA) School of Forest Resources, the University of Tennessee (UT) Department of Forestry, Wildlife and Fisheries, UT Botany Department, and the Tennessee Division of Forestry (TDF) was begun in 1987, when controlled pollinations were conducted by UT and TDF personnel among superior yellow-poplar selections at the University of Tennessee's yellow-poplar seed orchard near Knoxville, TN. Collections of developing aggregates of samaras were made at bi-weekly intervals throughout the summer and shipped via overnight mail to UGA. Breeding and staged collection of immature samaras were repeated in 1988. By initiating cultures from embryos obtained from the samaras at different developmental stages, it was ascertained that the optimal stage of embryo development for explanting to obtain embryogenic cultures was the globular to early heart stage, which occurred

approximately 8 weeks postpollination (Sotak et al., in press). In 1989, this information allowed us to make a single collection of control-pollinated seeds for culturing, resulting in production of the majority of the embryogenic lines used in this study.

Embryogenic yellow-poplar cultures were initiated following procedures outlined in Merkle and Sommer (1986). Briefly, following surface sterilization, samaras were dissected and immature zygotic embryos and endosperm were explanted onto a modified Blaydes (Witham et al. 1972) induction medium containing Murashige and Skoog's (1962) iron, Brown's minor salts (Sommer and Brown 1980), Gresshoff and Doy's (1972) vitamins, 40 g/l sucrose, 1 g/l casein hydrolysate (CH), 2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D), and 0.25 mg/l benzyladenine (BA). Usually within 2 months after explanting on induction medium, zygotic embryos produced proembryogenic masses (PEMs), which continued to proliferate as long as left on this medium. Embryogenic suspension cultures were initiated by inoculating PEMs into 50 ml Erlenmeyer flasks containing 20 ml of liquid induction medium. Suspension cultures were maintained on a gyratory shaker at 90 rpm.

Early attempts at somatic embryo production and conversion

When PEMs were transferred to solid or liquid basal medium (same as induction medium but lacking growth regulators), somatic embryos developed from them. However, the majority of these embryos failed to complete the normal sequence of development to the point where they resembled mature zygotic embryos. Instead, most were malformed, often with fused cotyledons. These malformed embryos became swollen, followed by radicle elongation (germination), callus formation or secondary embryo production. Germination was only rarely accompanied by apical development. Due to this pattern of development, overall conversion rates of yellow-poplar somatic embryos were below 1%. However, some emblings were produced by transferring well-formed embryos to a test tubes containing a Risser and White's (1964) plantlet development medium with 2% sucrose and no growth regulators. Following 2 months of growth on this medium, emblings were transferred to a peat/vermiculite potting mix, acclimatized to ambient conditions in a humidifying chamber and grown in the greenhouse. During 1988-90, approximately 450 potted yellow-poplar emblings derived from Tennessee material were delivered to UT and TDF cooperators. Three hundred of these emblings, along with 100 check seedlings were planted on the Ames Plantation, a UT Experiment Station near Memphis, TN, in March, 1991. Another 135 emblings were planted at the East Tennessee State Nursery (TDF) adjacent to a full-sib yellow-poplar seedling test in May, 1991. However, it was recognized that much larger populations of emblings would be needed to conduct a meaningful test.

Improvements in embryo conversion

In order to make the embryogenic cultures useful for mass propagation, conversion frequency had to be raised substantially. Treatments that were tested to improve conversion are described in detail in Merkle et al. (1990). One promising technique employing size fractionation and treatment with abscisic acid produced roughly synchronous populations of well-formed, apparently mature embryos in suspension culture. However, conversion rates of these suspension-cultured embryos remained below 1%. Therefore, an alternative protocol was

adopted in which suspension-cultured PEMs were size-fractionated and the desired fraction was immediately plated on semisolid medium (Figure 1).
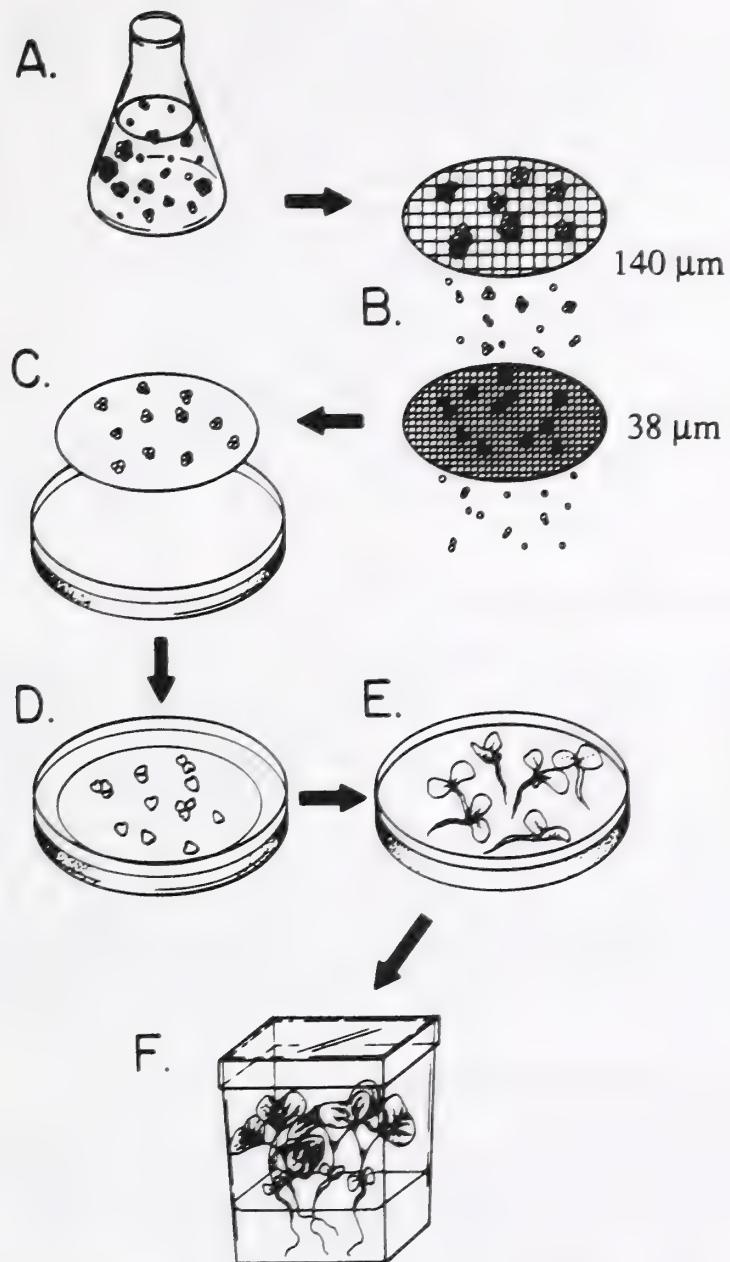
Figure 1. Fractionation/plating protocol used to produce yellow-poplar somatic embryos and emblings. A. Embryogenic suspensions are grown in liquid induction medium. B. PEMs are sieved on a 140 μm stainless steel screen and the fraction passing through is resieved on a 38 μm screen. C. PEMs remaining on the 38 μm screen are collected on filter paper and plated on basal medium. D. Somatic embryos develop synchronously from PEMs, maturing within 14 days. E. Mature embryos are transferred to basal medium without CH to promote germination. F. Germinants are transferred to plantlet development medium in GA7 vessels. (Figure from Parrott et al., in press).

In this protocol, two weeks following transfer to fresh induction medium, 1 g of PEMs was sieved on a 140 μm stainless steel screen and the fraction passing through was resieved on a 38 μm screen. The fraction remaining on the 38 μm screen was rinsed with basal medium and then backwashed from the screen onto a single layer of filter paper in a Buchner funnel. PEMs were again rinsed with basal medium while under mild vacuum, which served to spread PEMs into a single layer. When excess liquid medium had been drawn off, the filter paper with PEMs was placed on semisolid basal medium and incubated under fluorescent light (16 hr/day) at 30° C. As PEMs developed into somatic embryos on the filter paper, mature torpedo-stage embryos with well-developed cotyledons were selected and transferred to petri plates containing germination medium, which was the same as basal medium, but without CH. This modification of the basal medium was made following our discovery that CH inhibited somatic embryo germination, probably by raising the osmotic potential of the medium, and that eliminating it promoted vigorous germination and cotyledon greening within 1 week (Merkle et al. 1990). Using this method, a 60-70% synchronous population of embryos could be produced within 2 weeks following plating of PEMs, and following transfer to plantlet development medium, an average of 32% of these somatic embryos converted to emblings (Merkle et al. 1990).

Scale-up and somatic embryo production for field testing

Approximately 20 embryogenic suspension cultures were tested for ability to produce embryos and emblings. Based on data from this test, 9 embryogenic clones were chosen for scale-up. The selected clones displayed mature embryo conversion rates of up to 100% and overall conversion rates of up to 85% (Table 1). Amount of PEMs available for embryo production from a clone at a given time was increased by growing suspensions of the selected lines in 80 ml of induction medium in 250 ml Erlenmeyer flasks on a gyratory shaker. Cultures were maintained by inoculating fresh medium with approximately 2 g of PEMs every 3 weeks. Synchronous populations of embryos of the 9 clones chosen for scale-up were produced using the same size fractionation/plating method described above. Mature embryos were transferred to germination medium in 60 x 15 mm petri plates, 25 embryos per plate.

Plantlet development and acclimatization

Plates of embryos on germination medium were carefully packed and shipped via overnight mail to cooperators at UT. During February and March, 1990, over 1000 embryos of each of the 9 clones were produced and shipped. Upon arrival at UT, germinants were aseptically transferred to GA7 vessels (Magenta Corp.) containing 100 ml of semisolid plantlet development medium. Twenty-five germinants were "planted" in each GA7 vessel and grown under fluorescent light at 22° C, where they completed conversion to emblings. Following 2-3 months of growth in GA7 vessels, emblings were removed from *in vitro* conditions and transferred fir cell Cone-tainers™ containing a peat/vermiculite potting mix. Previously, when somatic embryo-derived emblings were hardened-off at UGA, numbers were small enough so that acclimatization could be handled in a small chamber where humidity was maintained by an atomizer. However, the acclimatization of thousands of emblings required a much larger facility with controlled humidity.

Table 1. Somatic embryo production and conversion performance for Tennessee clones to be used in field test

Clone	Embryos/g PEMs (S.E.)	Mature/g PEMs (S.E.)	Conversion Mature	Conversion Overall	Emblings/g PEMs
14X108	850 (430)	720 (390)	88	74	630
1X10	370 (50)	220 (30)	88	52	190
10X1	850 (180)	740 (190)	96	84	710
4X12	950 (240)	720 (230)	58	44	420
4X10	350 (140)	230 (70)	96	63	220
5X39	220 (20)	150 (40)	100	68	150
5X7	350 (20)	310 (20)	96	85	300
7X14	800 (110)	630 (140)	64	50	400
10X4	280 (60)	200 (70)	100	71	200
Mean	560 (90)	440 (80)	87	66	360

Therefore, a 25 X 25 ft greenhouse bay at UT was modified by installing a fogging system to maintain various levels of humidity. Transfer of emblings from GA7 vessels to potting mix was performed in the greenhouse with the fogging system running to prevent desiccation of the emblings. Emblings were grown under fog for 2-3 months, during which time they were fertilized periodically with commercial plant fertilizer. Beginning approximately 2 months following initial potting, emblings that had produced new leaves and extensive root systems were transplanted to 45 cu.in. root-trainers containing the same potting mix, and transferred to a standard greenhouse. Root-trainers were not used initially due to limited space in the fogging bay of the greenhouse. Transplanting continued into early October.

During acclimatization using the fogging system at UT, yellow-poplar emblings failed to display the vigorous growth we had observed when emblings were acclimatized using the atomizer-driven humidifying chamber at UGA. Although an experiment is currently underway to determine the cause of the slow growth, it is likely that uneven distribution of moisture by the fogging system resulted in excessive moisture being deposited on developing leaves of some emblings, while others did not receive sufficient moisture. Nevertheless, of the approximately 8700 emblings transferred to *ex vitro* conditions, approximately 5500 (63%) were successfully hardened-off and transferred to the shadehouse,

where they set buds during autumn, 1990. Over 99% of these emblings successfully overwintered and broke dormancy during April, 1991, continuing seedling-like growth and development. At this stage, well-defined phenotypic differences could be observed among clonal blocks of emblings, similar to those seen among blocks of full-sibling seedling families. Current plans call for these emblings to be grown for another season in the shadehouse and planted in the field during early spring, 1992, on several UT Experiment Station sites. Full-sibling seedlings of some of the same families will be included as check plots.

<u>Labor commitment</u>

The various activities in the mass propagation effort were carefully monitored to aid in estimating labor commitments for this type of research. The production of approximately 12,000 somatic embryos by the UGA laboratory required about 150 person-hours. At UT, transferring the germinants to GA7 vessels and subsequently from GA7 vessels to Cone-tainers was estimated at 265 person-hours. An additional 130 person-hours were committed to transplant emblings from Cone-tainers to root-trainers, making the total labor commitment 545 person-hours. Dividing this number by the total number of emblings in soil generated by the experiment produces a statistic of 6 minutes of labor per embling. Field establishment of yellow-poplar emblings is projected to take 9 minutes per embling, based on estimates obtained in spring, 1991, for site preparation and planting full-sibling yellow-poplar seedling genetic tests. Therefore the total labor commitment for expansion of existing embryogenic cultures for mass production of somatic embryos to field establishment of emblings will be approximately 15 minutes per embling. This figure does not include time allocated for breeding activities, initial culture establishment or greenhouse/shadehouse maintenance.

Two factors may decrease the labor/embling estimate. Restricted greenhouse space dictated the use of Cone-tainers for the acclimatization process. The 3 cu.in. Cone-tainers employed were too small to grow yellow-poplar emblings to the size required for field establishment, necessitating transfer of the emblings to 45 cu.in. root-trainers. This transplanting step, requiring 130 person-hours, can be eliminated given sufficient greenhouse space, reducing the laboratory-through-acclimatization labor from 6 minutes per embling to 4.5 minutes per embling. In addition, we believe the percentage of emblings successfully hardened-off to greenhouse conditions could realistically be raised to at least 80%, based on results obtained using the atomizer-driven humidifying chamber at UGA. Based on this higher success rate, labor could be reduced to 3.6 minutes/embling. This estimate approaches the time required to transplant a yellow-poplar seedling from a germination tray to root-trainer for containerized production.

FUTURE FIELD TEST PROJECTS

Although the current experiment has demonstrated large scale clonal propagation of yellow-poplar is possible using somatic embryos, data on the actual field performance of these trees will not be available for some years. Furthermore, because the embryogenic cultures used in this experiment were originally initiated for other purposes, our design was limited and did not include a number of critical features needed to determine the true potential of

these propagules to substitute for seedlings. A major problem was the unavailability of yellow-poplar seedlings of the same age as the somatic embryo-derived trees from each of the families from which the embryogenic cultures were derived. Thus, we could not make quantitative comparisons of field performance between the two types of propagules. Another consideration is the fact that the cultures from which the emblings were derived were cultures that were initiated in 1987, 1988 and 1989. Thus some of the lines were over 3 years old when embryos were produced from them, while others were less than one year old. As embryogenic cultures become habituated to growth in suspension, somatic embryos capable of conversion often are produced at lower frequencies. It is also likely that such characteristics as clonal fidelity of emblings deteriorate over time. Therefore, we do not believe that a rigorous quantitative test of field performance of yellow-poplar emblings can be accomplished with the available material.

To lay the foundation for a test that will accomplish the goals outlined in the introduction, we have designed and initiated a new study. Controlled pollinations conducted in the UT yellow-poplar seed orchard during May, 1991 will provide seeds with which to initiate embryogenic cultures and seedling populations from at least 5 full-sib families. We expect to obtain at least 4 embryogenic cultures from each family for a total of 20 embryogenic lines. Synchronous populations of somatic embryos will be produced from each line, germinated, transferred to pots and acclimatized as described above. Emblings will be established in replicated, incomplete block field planting designs, along with seedlings from the same 5 full-sib families. Ten-tree plots will be used and each treatment (clonal line or seedling family) will be represented by 4 replications. This experimental design is intended to answer the following questions: (1) How stable is the performance of emblings within individual clones versus that of seedlings within individual families?, and (2) Overall, are emblings significantly different from seedlings in performance?

CONCLUSIONS

Although embryogenic regeneration systems for forest trees are expected to become a major source of clonal material for mass propagation and gene transfer purposes, currently very few systems are capable of producing large numbers of usable propagules for these applications. Once these systems begin to reach their potential, a major question will be how well trees derived from somatic embryos fulfill their role in providing plantations of superior trees. We have demonstrated that a collaborative research program between plant tissue culture laboratories and applied tree improvement programs can be established to begin to provide the answer to this question. Preliminary results from our project indicate that production-scale clonal propagation of hardwood forest trees is possible by combining traditional tree breeding, a high frequency embryogenic system and a standard hardening-off treatment. Whether or not the resulting emblings will provide a viable alternative to seedlings will not be known for some years.

LITERATURE CITED

Ahuja, M.R. 1987. *In vitro* propagation of poplar and aspen. In Cell and Tissue Culture in Forestry, Vol. 3, J.M. Bonga and D.J. Durzan (eds.). Martinus Nijhoff Publishers, Dordrecht. pp. 234-246.

Amerson, H.V., L.J. Frampton, Jr., R.L. Mott, and P.C. Spaine. 1988. Tissue culture of conifers using loblolly pine as a model. In Genetic Manipulation of Woody Plants, J.W. Hanover and D.E. Keathley (eds.). Plenum Publishing, New York. pp. 117-137.

Becwar, M.R., T.L. Noland, and J.L. Wyckoff. 1989. Maturation, germination, and conversion of Norway spruce (*Picea abies* L.) somatic embryos to plants. In Vitro Cell. Dev. Biol. 25:575-580.

Boulay, M. 1989. Redwood (*Sequoia sempervirens*). In Biotechnology in Forestry and Agriculture, Vol. 5. Trees II, Y.P.S. Bajaj (ed.). Springer-Verlag, Berlin. pp. 549-573.

Chalupa, V. 1987. European hardwoods. In Cell and Tissue Culture in Forestry, Vol. 3, J.M. Bonga and D.J. Durzan (eds.). Martinus Nijhoff Publishers, Dordrecht. pp. 234-246.

Choo, W.K. 1990. Oil palm (*Elaeis guineensis* Jacq.): tissue culture and micropropagation. In Biotechnology in Forestry and Agriculture, Vol. 10. Legumes and Oilseed Crops I, Y.P.S. Bajaj (ed.). Springer-Verlag, Berlin. pp. 569-592.

Corley, R.H.V., C.Y. Wong, K.C. Wooi, and L.H. Jones. 1981. Early results from the first oil palm trials. In The Oil Palm in Agriculture in the Eighties, E. Pushparajah and P.S. Chew (eds.). Inc. Soc. Planters, Kuala Lumpur, Malaysia. pp. 173-196.

Finer, J.J., and M.D. McMullin. 1990. Transformation of cotton (*Gossypium hirsutum* L.) via particle bombardment. Plant Cell Rep.8:586-589

Frampton, L.J., R.L. Mott, and H.V. Amerson. 1985. Field performance of loblolly pine tissue culture plantlets. In Proceedings of the 18th Southern Forest Tree Improvement Conference. pp. 136-144.

Gordon-Kamm, W.J., T.M. Spencer, M.L. Mangano, T.R. Adams, R.J. Daines, W.G. Start, J.V. O'Brien, S.A. Chambers, W.R. Adams, Jr., N.G. Willets, T.B. Rice, C. J. Mackey, R.W. Krueger, A.P. Kausch, and P.G. Lemaux. 1990. Transformation of maize cells and regeneration of fertile transgenic plants. The Plant Cell 2:603-618.

Gresshoff, P.M., and C.H. Doy. 1972. Development and differentiation of haploid *Lycopersicon esculentum* (tomato). Planta 107:161-170.

Gupta, P.K., and D.J. Durzan. 1987. Biotechnology of somatic polyembryogenesis and plantlet regeneration in loblolly pine. Bio/technology 5:147-151.

Kitto, S., and J. Janick. 1985. Production of synthetic seeds by encapsulating asexual embryos of carrot. J. Amer. Soc. Hort. Sci. 110:277-282.

Krogstrup, P. 1990. Effect of culture densities on cell proliferation and regeneration from embryogenic cell suspensions of *Picea sitchensis*. Plant Sci. 72:115-123.

McCown, D.D., and B.H. McCown. 1987. North American hardwoods. In Cell and Tissue Culture in Forestry, Vol. 3., J.M. Bonga and D.J. Durzan (eds.). Martinus Nijhoff Publishers, Dordrecht. pp. 247-260.

McGranahan, G.H., C.A. Leslie, S. Uratsu, L.A. Martin, and A.M. Dandekar. 1988. *Agrobacterium*-mediated transformation of walnut somatic embryos and regeneration of transgenic plants. Bio/technology 6:800-804.

McGranahan, G.H., C.A. Leslie, S.L. Uratsu, and A.M. Dandekar. 1990. Improved efficiency of the walnut somatic embryo gene transfer system. Plant Cell Rep. 8:512-516.

Mascarenhas, A.F., S.V. Kendurkar, P.K. Gupta, S.S. Khuspe, and D.C. Agrawal. 1987. Teak. In Cell and Tissue Culture in Forestry, Vol. 3, J.M. Bonga and D.J. Durzan (eds.). Martinus Nijhoff Publishers, Dordrecht. pp. 300-315.

Merkle, S.A., and H.E. Sommer. 1986. Somatic embryogenesis in tissue cultures of *Liriodendron tulipifera*. Can. J. For. Res. 16:420-422.

Merkle, S.A., A.T. Wiecko, R.J. Sotak, and H.E. Sommer. 1990. Maturation and conversion of *Liriodendron tulipifera* somatic embryos. In Vitro Cell. Dev. Biol. 26:1086-1093.

Murashige, T., and F. Skoog. 1962. A revised medium for rapid growth and bioassays with tissue cultures. Physiol. Plant. 15:473-497.

Redenbaugh, K., B.D. Paasch, J.W. Nichol, M.E. Kossler, P.R. Viss, and K.A. Walker. 1986. Somatic seeds: encapsulation of asexual plant embryos. Bio/technology 4:797-801

Risser, P.G., and P.R. White. 1964. Nutritional requirements of spruce tumor cells *in vitro*. Physiol. Plant. 15:620-635.

Ritchie, G.A., and A.J. Long. 1986. Field performance of micropropagated Douglas fir. N.Z. J. For. Sci. 16:343-356.

Parrott, W.A., S.A. Merkle, and E.G. Williams. 1991. Somatic embryogenesis: potential for use in propagation and gene transfer systems. In Advanced Methods in Plant Breeding and Biotechnology, D.R. Murray (ed.). CAB International, Wallingford, Oxon (UK).

Roberts, D.R., B.C.S. Sutton, and B.S. Flinn. 1990. Synchronous and high frequency germination of interior spruce somatic embryos following partial drying at high relative humidity. Can. J. Bot. 68:1086-1090.

Shillito, R.D., G.K. Carswell, C.M. Johnson, J.J. DiMaio, and C.T. Harms. 1989. Regeneration of fertile plants from protoplasts of inbred maize. Bio/technology 7:581-587.

Sommer, H.E., and C.L. Brown. 1980. Embryogenesis in tissue cultures of sweetgum. For. Sci. 26:257-260.

Sommer, H.E., H.Y. Wetzstein, and N. Lee. 1985. Tissue culture of sweetgum (*Liquidambar styraciflua* L.). In Proceedings of the 18th Southern Forest Tree Improvement Conference. pp. 42-50.

Sotak, R.J., H.E. Sommer, and S.A. Merkle. Relation of the developmental stage of zygotic embryos of yellow-poplar to their somatic embryogenic potential. Plant Cell. Rep. (in press).

Stuart, D.A., S.G. Strickland, and K.A. Walker. 1987. Bioreactor production of alfalfa somatic embryos. HortScience 22:800-803.

Styer, D.J. 1985. Bioreactor technology for plant propagation. pp. 117-123. In: R.R. Henke, K.W. Hughes, M.J. Constantin, and A. Hollaender (eds.) Tissue Culture in Forestry and Agriculture. Plenum Press, New York/London.

Thorpe, T.A., I.S. Harry, and P.P. Kumar. Application of micropropagation to forestry. In Micropropagation Technology and Application, P.C. Debergh and R.H. Zimmerman (eds.). Kluwer Academic Publishers, Dordrecht. pp. 311-336.

Tulecke, W. 1987. Somatic embryogenesis in woody perennials. In Cell and Tissue Culture in Forestry, Vol. 2, J.M. Bonga and D.J. Durzan (eds.). Martinus Nijhoff Publishers, Dordrecht. pp. 61-91.

Wann, S.R. 1989. Somatic embryogenesis in woody species. In Horticultural Reviews, Vol. 10, J. Janick (ed.). Timber Press, Portland. pp. 153-181.

Webster, F.B., D.R. Roberts, S.M. McInnis, and B.C.S. Sutton. 1990. Propagation of interior spruce by somatic embryogenesis. Can. J. For. Res. 20:1759-1765.

Witham, F.H., D.F. Blaydes, and R.M. Devlin. 1971. Experiments in Plant Physiology. Van Nostrand-Reinhold Co., New York. 245 p.

Use of Proteinase Inhibitors for Crop Protection

S. Park and R. W. Thornburg[1]

Abstract -- Proteinase inhibitors are generally small proteins that specifically inhibit the action of proteinases. They are produced by plants in great quantities, yet they have no known function within plant tissues. They have been hypothesized to specifically interact with insect proteinases to protect plants against insect attack. Proteinase inhibitors accumulate in the seeds or storage organs of all plants, however, in the solanaceae, proteinase inhibitors also accumulate in the foliage of these plants. Further, they are normally expressed in the foliage at low levels, but following attack by insects, the levels of proteinase inhibitors increase dramatically.

We have isolated from a Russet Burbank Potato DNA genomic library, several genes coding for proteinase Inhibitor II. These genes have been analyzed at the molecular and functional level. Characterization of these genes has increased our understanding of the function of these proteinase inhibitors in plant tissues. The proteinase inhibitors can interact with a variety of proteinases, but the Inhibitor II's that we have isolated are specific for both trypsin and chymotrypsin.

We have prepared chimeric genes that express marker genes under the control of the wound-inducible Proteinase Inhibitor II promoter. These chimeric genes have been used to transform both tobacco plants and poplar trees. The wound-induction of these chimeric genes in the transgenic plants is similar to the induction of the genes in wild-type potatoes and tomatoes. When transgenic plants are placed in the field, the plants are fully capable of responding to insect attack by inducing new marker protein synthesis.

---

[1]Department of Biochemistry and Biophysics, Iowa State University, Ames, IA 50011   (515) 294-7885.

INTRODUCTION

Plant proteinase inhibitors were first recognized in wheat flour 54 years ago by Read and Haas (1938). Since that time, many researchers have considered possible roles of proteinase inhibitors in plants. Early work considered plant proteinase inhibitors as possible regulatory proteins or storage proteins. However, the function of proteinase inhibitors as a regulator of endogenous proteinases was questioned because Ofelt et.al. (1955) showed that soybean proteinase inhibitors did not inhibit endogenous soybean proteinase and this conclusion was affirmed by Birk and Waldman (1965). Therefore, the storage role for the inhibitors was supported by their presence in large quantities in seeds and tubers. For example, the inhibitors are present in about 6% of the total soybean proteins (Rackis and Anderson, 1964) and up to 10% of the soluble proteins of potato tubers (Ryan et.al., 1968a).

Interest in plant proteinase inhibitors has expanded from earlier research on their regulatory or storage roles in plant and effects in the human food chain to more recent interest in their possible contributions to natural protection systems of plants. In 1964, Applebaum first proposed the role of plant proteinase inhibitors as a defense mechanism against insects based upon the study on the effect of soybean trypsin inhibitor on legume beetles (Applebaum, 1964). Moses Kunitz and co-workers established that trypsin inhibition by trypsin inhibitor was a result of a protein-protein association between the enzyme and inhibitor to form a complex that blocks trypsin activity (Kunitz and Northrop, 1936, Kunitz, 1947a and 1947b).

Potato Proteinase Inhibitors

In addition to the soybean trypsin inhibitor, other proteinase inhibitors have also received much attention. The chymotrypsin inhibitor from potato tubers (Ryan and Ball, 1962, Ball and Ryan, 1963), called Inhibitor I (Ryan, 1968a), was found to have inhibitory activity against proteinases from mammalian, bacterial, and fungal origins not from plant origin (Ryan, 1966). The potato Inhibitor I was shown to consist of four subunits and the molecular weight of Inhibitor I was found to be 39,000 ± 2,000 and that of its complex saturated with chymotrypsin, 140,000 ± 4,600, (Melville and Ryan, 1972), indicating that four molecules of chymotrypsin could make a complex with one molecule of Inhibitor I. Inhibitor I also was found in the detached potato (Ryan, 1968a) and tomato (Ryan, 1968b) leaves, which were incubated in water, but not in the attached leaves.

The possible involvement of proteinase inhibitors in plant protection received considerable support with the discovery in 1972, that attack of Colorado potato beetles on potato and tomato plants induced the accumulation of proteinase inhibitors in the leaves (Green and Ryan, 1972). This accumulation occurred even in leaves distant from the attack sites.

After this discovery, research focused on the characterization of proteinase inhibitor proteins as well as the chemical signals that induce the proteinase inhibitor accumulation. Several proteinase inhibitors are found in tomato and potato. The most thoroughly characterized of these inhibitors have been Inhibitor I (Melville and Ryan, 1972, Richardson, 1974, Richardson and Cossins, 1974) and Inhibitor II (Gustafson and Ryan, 1976, Bryant et.al.,1976).

The tomato inhibitors were shown to be highly homologous with potato inhibitors since tomato Inhibitors I and II strongly cross-reacted with antibodies prepared against each respective potato inhibitors (Gustafson and Ryan, 1976). Inhibitors I and II were also purified from wounded tomato leaves and were shown to be very similar to potato tuber Inhibitors I and II in subunit molecular weight, composition, and inhibitor activities (Plunkett et.al., 1982).

In an effort to understand the function and significance of the proteinase Inhibitor II gene family, we have isolated a series of proteinase inhibitor genes from a potato genomic library. The first of these proteinase Inhibitor II genes was previously isolated and characterized (Thornburg, et al., 1987). We have since isolated and characterized a second Proteinase Inhibitor II gene from the potato library (Park, 1991). Some of the general characteristics of the proteinase inhibitor genes are presented below.

Characteristics of Potato Inhibitor II genes

In general, the Inhibitor II open reading frame is composed of two exons separated by a single small intron. The intron-exon junctions obey the GT-AG rule (Breathnach, et al., 1978, Brown, 1986), in which intron sequences usually start with GT and end with AG.

The Inhibitor II protein is synthesized as a preprotein and the signal sequence targets the vacuolar membrane to transport the mature Inhibitor II protein into the vacuole (Nelson and Ryan, 1980). The Inhibitor II genes are processed during or shortly after synthesis between amino acid residues 25 and 26 to produce the mature inhibitor. The deduced amino acid sequence of *pin2T* indicates that it is initially translated with a

sequence of 147 amino acids, which is 7 amino acid shorter than that of the previously characterized *pin2K*. Of the 147 amino acids, the first 25 translated amino acids (30 amino acids in *pin2K*), apparently function as a signal sequence (von Heijne, 1983) to facilitate transport of the mature protein into the vacuole where it is stored until it is needed to combat insect attack (Walker-Simmons and Ryan, 1977). The signal sequence maintains cleavage specificity with the "-3/-1" rule (von Heijne, 1983), in which the signal sequence has small, neutral amino acid residues in positions -3 and -1 (counting from the cleavage site between positions -1 and +1) but are rare in -2. In the case of the Inhibitor IIT open reading frame, Ala and Val are located in positions -1 and -3, respectively. In addition to this (-3, -1) rule, the signal sequence of Inhibitor IIT contain charged residues near both termini of its sequence (in positions 5, 6, and 24) and an extended hydrophobic core. Therefore, signal sequence of Inhibitor IIT seems to be fit very well to the general properties of the known signal peptide. Hydropathy plots (Kyte and Doolittle, 1982) of the signal sequence of the Inhibitor IIT protein clearly shows that the amino terminal segment is rich in hydrophobic amino acid resides.

The entire pre-Inhibitor IIT amino acid sequence exhibits around 80% identity with deduced amino acid sequences from other Inhibitor II genes. The major difference lies in the length of the signal sequence. The mature proteinase inhibitors are very similar. Figure 1 shows the comparison of the deduced amino acid sequences of the potato Inhibitor IIT with those of the other Inhibitor II proteins.

```
                    10        20        30 |    40        50
PI-IIT    MAVHKEVSFVAYLLIVLGMFLY    VDALGCTKECGNLGFGICPRSEGS
T247      MAVHKEVNFVAYLLIVLGMFLY    VDAKACTRECGNLGFGICPRSEGS
PI-IIK    MDVHKEVNFVAYLLLVLGLLVLVSAMD VDAKACIRECGNLGFGICPRSEGS
PI-II     MDVHKEVNFVAYLLIVLGLLVLVSAMEHVDAKACTLECGNLGFGICPRSEGS
CDNA 1    MDVHKEVNFVAYLLIVLGLLVLVSAMEHVDAKACTLECGNLGFGICPRSEGS
GTI2-P    MAVHKQVSFLAYLLLVLGLLLLLVSAVEHVDAKPCTLECGHLGFGICPRSEGS
                                        P₁(Domain I)

               60        70        80        90        100
PI-IIT    PTNPICINCCSGYKGCNYYSAFGRFICEGESDPKNPKACPLNCDTNIAYSRC
T247      PLNPICINCCSGYKGCNYYNSFGKFICEGESDPKRPHACTFNCDPNIAYSRC
PI-IIK    PENPICTNCCAGYKGCNYYSANGAFICEGQSDPKKPKACPLNCDPHIAYSKC
PI-II     PENRICTNCCAGYKGCNYYSANGAFICEGQSDPKKPKACPLNCDPHIAYSKC
CDNA 1    PENPICTNCCAGYKGCNYYSANGAFICEGQSDPKKPKACPLNCDPHIAYSKC
GTI2-P    PQNPICTNCCAGFKGCNYYSAHGTFICEGQSDPRNPKACPRNCDPHIAYSKC
                                        P₁(Domain II)

               110       120       130       140       150
PI-IIT    RCPRSEGKSLIYPTGCTTCCTGYKGCYYFGTNGKFVCEGESDSPKPYMSTA
T247      RCPRSQGKSLIYPTGCTTCCTGYKGCYYFGKDGKFVCEGESDEPKANMYPVM
PI-IIK    KCPRSEGKSLIYPTGCTTCCTGYKGCYYFGKDGKFVCEGESDEPKGNMYPVM
PI-II     KCPRSEGKSLIYPTGCTTCCTGYKGCYYFGKNGKFVCEGESDEPKANMYPAM
CDNA 1    KCPRSEGKSLIYPTGCTTCCTGYKGCYYFSKNGKFVCEGESDEPKANMYPAM
GTI2-P    KCPRSEGKTFIYPTGCTTCCTGYKGCYYFGKDGKFVCEGESDEPKVCYYPGM
```

Figure 1. Comparison of the deduced amino acid sequence of the Potato Inhibitor II T with those of the other Inhibitor II genes. The single letter amino acid code is used. The identity between the inhibitors is indicated by bold face. The putative cleavage site of the transit sequence is shown with an arrow. Gaps were introduced for best fit of alignment in the transit sequence. Active site at P_1 is shown by an asterisk in both domains. All of the 16 cysteine residues are underlined. The amino acid sequences of the T_247, PI-IIK, PI-II, cDNA1 and GTI2-P are from Graham, et al., 1985; Thornburg, et al., 1987; Keil, et al., 1986; Sanchez-Serrano, et al., 1986; and Fox, 1986, respectively.

Potato Inhibitor IIT protein, like other Inhibitor II proteins, consists of two domains that share 50% amino acid identity. However, these domains differ in their active site P1-P1' residues (Schechter and Berger, 1967). The active site P1-P1' at amino acid residues 30-31 of the Inhibitor IIT protein for domain 1, is Lys-Glu, and amino acid residues 87-88 for domain 2, Leu-Asn. Figure 2 shows the comparison of internal homology within the Inhibitor IIT amino acid sequence. The boxed regions indicate the amino acid identity with each other in two domains.
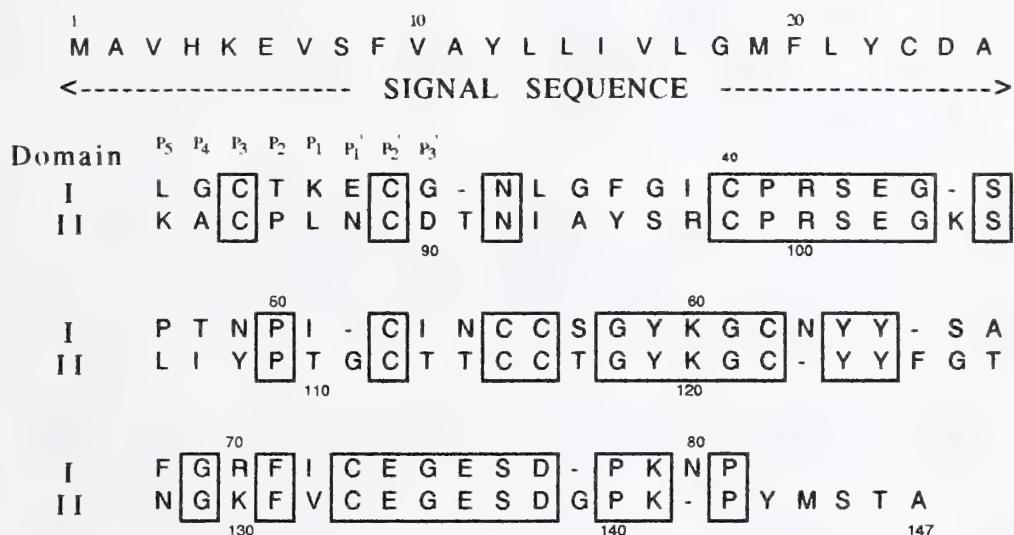
Figure 2. Alignment of the trypsin domain (Domain I) with the chymotrypsin domain (Domain II) of the Inhibitor IIT protein sequence. The single letter amino acid code is used throughout. The signal sequence is shown above the alignment of the two domains. The amino acid sequences are aligned such that the conserved cysteine residues show maximum homology. Gaps were introduced for best fit of alignment. The N-terminal domain is labeled Domain I while the C-terminal domain is labeled Domain II. The boxed regions indicate the homologous sequence between two domains. Labeling of the inhibitory site amino acid residues is indicated (P5-P'3).

The common rules for inhibitory sites have been proposed by Kowalski and Laskowski (1972) in which inhibitors with P1 Lys and Arg tend to inhibit trypsin and trypsin-like enzymes, and those with P1 Tyr, Phe, Leu and Met inhibit chymotrypsin and chymotrypsin-like enzymes. According to the common rules for inhibitory sites, the site in domain 1 would be specific for trypsin-like proteases, while in domain 2, the reactive site is specific for chymotrypsin-like proteases. This "double headedness" is typical for members of both the Inhibitor II family (Ryan and Hass, 1980) and the Bowman-Birk family (Ikenaka and Odani, 1978) of proteinase inhibitors from plants. Both the Inhibitor II and Bowman-

Birk families contain two inhibitory domains, although these two families are unrelated.

Potato Inhibitor II family, which is one of 13 different families of serine proteinase inhibitors in nature (Laskowski, 1986), is known to inhibit both chymotrypsin-like and trypsin-like proteins from either animal or microbial origin (Ryan, 1973). The comparison of the reactive sites in potato Inhibitor II family (Table 1) indicated that three subgroups are present in this family. They are Inhibitor II which has two trypsin-specific domains, Inhibitor II which has two chymotrypsin-specific domains, and Inhibitor II which has one trypsin-specific domain and one chymotrypsin-specific domain. Inhibitors IIT and IIK genomic clones of potato and tomato Inhibitor II cDNA contain one trypsin-specific domain and one chymotrypsin-specific domain. Tomato Inhibitor II genomic clone contains two trypsin-specific domains. Another potato Inhibitor II cDNA and genomic clones contain two chymotrypsin-specific domains. In fact, isoforms of Inhibitor II which has two trypsin-specific domains have been isolated in Dr. Clarence A. Ryan's laboratory (Washington State University) (Fox, 1986). This result supports the presence of three subgroups in the potato Inhibitor II family.

The potato Inhibitor II gene codes for a protein which shares high homology with two small molecular weight polypeptides that were isolated from potato tubers (Hass et.al., 1982). The potato polypeptide trypsin inhibitor, PTI (MW. 5,100), exhibits 82% amino acid homology with the middle sequence of the Inhibitor IIT protein, whereas the potato polypeptide chymotrypsin inhibitor, PCI (MW. 5,400), shows 84% homology with the Inhibitor IIT protein. Figure 3 shows the alignment of the deduced amino acid sequence of *pin2T* with the amino acid sequences of PTI and PCI. PTI and PCI do not dimerize like Inhibitor II and thus exist as single

```
PI-II T  MAVHKEVSFVAYLLIVLGMFLYVDALGCTKECGNLGFGIC
                                       Inhibitory
                                         site

PI-II T  PRSEGSPTNPICINCCSGYKGCNYYSAFGRFICEGESDPK
                  *** ***  **********  *  *****  ****
PCI               PICTNCCAGYKGCNYYSANGAFICEGQSDPK
                  *************************  ****
PTI               RICINCCSGYKGCNYYSAFGRFICEGESDPK


PI-II T  NPKACPLNCDTNIAYSRCPRSEGKSLIYPTGCTTCCTGYK
         *********  ****  ****
PCI      KPKACPLNCDPHIAYSKCPRS
          *  ** ***  ******  **
PTI      NPNVCPRNCDTNIAYSKCLR
            Inhibitory
              site


PI-II T  GCYYFGTNGKFVCEGESDEPKPYMSTA#
```

Figure 3. Alignment of the deduced amino acid sequence of *pin2T* with the amino acid sequences of polypeptide trypsin inhibitor (PTI) and polypeptide chymotrypsin inhibitor (PCI). The single letter amino acid code is used throughout. The identity between the two inhibitors is indicated by asterisks. The symbol, #, represents the stop codon of the Inhibitor IIT protein.

monomeric subunits (Pearce et.al., 1982). The PCI and PTI, represent only half the sequence of the Inhibitor II. They contain only a single inhibitory site. (amino acid 38). In both small inhibitors, the active site corresponds to the second active site (amino acid 87) of the full length Inhibitor IIT. It is thought that PCI and PTI may be derived from the Inhibitor II molecules by the action of plant proteinases.

Use of Proteinase Inhibitors to protect Crop Plants

Several studies have previously been performed indicating that the levels of proteinase inhibitors in plant tissues are correlated with insect resistance (Gatehouse and Boulter, 1983; Broadway, et al., 1986). Because of the ability of the proteinase inhibitors to effectively block the action of digestive proteases of animals, but not of plants, the proteinase inhibitors have been proposed as components of plant defenses that could be transferred from one species to another.

Much of the pioneering work that has been done in this area has been done in easily manipulated plants such as tobacco and tomato. In the first studies transgenic tobacco plants were constructed that contained the cowpea trypsin inhibitor under the control of the constitutive CaMV 35S promoter (Hilder, et al., 1987). When larvae of the tobacco budworm (*Heliothis virescens*) were placed on the plants that expressed the cowpea trypsin inhibitor at significant levels, there was reduced leaf damage on the transgenic plants relative to untransformed controls. This reduction in the leaf damage also was correlated with the level of inhibitor present in the tissues. In other recent studies, Johnson et al., (1990) demonstrated that the potato Proteinase Inhibitor II also can function to limit insect growth in transgenic tobacco plants. The transfer of these proteinase inhibitors to a wide variety of crop species is currently underway in a number of laboratories, where the inhibitors should provide plants with antinutrient properties thereby preventing herbivorous insects or fungi from deriving proper nourishment from the plant tissues. Transfer of such genes into trees has yet to be confirmed, but the success of the tobacco experiments indicate that similar results could be obtained in woody species (Thornburg, 1990).

In addition, the wound-inducible phenotype of the proteinase inhibitor gene system (Thornburg, et al., 1987) is another aspect about these genes that could be used for agricultural purposes in transgenic plants. It is known from field studies of transgenic plants, both tobacco (Thornburg, et al., 1990; Thornburg, 1991) and poplar trees (Klopfenstein, et al., 1991; McNabb, et al., 1991) that the proteinase inhibitor promoter is capable of expressing novel chimeric genes in response to insect attack, and it is further known that the Inhibitor II promoter induces these

chimeric genes in precisely those tissues that the insect preferentially consume (Thornburg, et al., 1990).

LITERATURE CITED

Applebaum, S.W. (1964) Physiological aspects of host specificity in the Bruchidae-I. General considerations of developmental compatibility. *J. Ins. Physiol.* **10**:783-788.

Ball, A. K. and Ryan, C.A. (1963) Concerning a crystalline chymotryptic inhibitor from potatoes, and its binding capacity for the enzyme. *J. Biol. Chem.* **238**:2976-2982.

Birk, Y. and Walman, M. (1965) Amylolytic-, trypsin-inhibiting-, and urease-activity in three varieties of soybeans and in soybean plant. Qual. Plat. Mater. Veg. **12**:199-209.

Breathnach, R., Benoist, C., O'Hare, K., Gannon, F. and Chambon, P. (1978) Ovalbumin gene: Evidence for a leader sequence in mRNA and DNA sequences at the intron-exon boundaries. Proc. Natl. Acad. Sci. USA. **75**:4853-4857.

Broadway, R., M., Duffey, S.S., Pearce, G. and Ryan, C.A. (1986) Plant proteinase inhibitors: A defense against herbivorous insects? Entomol. exp. appl. **41**:33-38.

Brown, J. W.. (1986) A catalogue of splice junction and putative branch point sequences from plant introns. Nucleic Acids Res. **14**:9549-9559.

Bryant, J., Green, T. R., Gurusaddaiah, T., and Ryan, C.A. (1976) Proteinase inhibitor II from potatoes: Isolation and characterization of its protomer components. Biochemistry **15**:3418-3423.

Fox, E.A. (1986) Isolation and characterization of a proteinase inhibitor II gene from Lycopersicon esculentum. PhD Dissertation, Washington State University.

Gatehouse, A.M.R. and Boulter, D. (1983) Assessment of the anti-metabolic effects of trypsin inhibitors from cowpea (Vigna unguiculata) and other legumes on development of bruchid beetle (Callosobruchus maculatus). **34**:345-350.

Graham, J., Pearce, G., Merryweather, J., Titani, K., Ericsson, L. and Ryan, C. (1985) Wound-induced proteinase inhibitors from tomato-leaves. II. The cDNA-deduced primary structure of pre-inhibitor II. J. Biol. Chem. **260**:6561-6564.

Green, J. S. and Ryan, C.A. (1972) Wound-induced proteinase inhibitor in plant leaves: A possible defense mechanism against insects. *Science* **175**:776-777.

Gustafson, G and Ryan, C.A. (1976) Specificity of protein turnover in tomato leaves. J. Biol. Chem. **251**:7004-7010.

Hilder, V.A., Gatehouse, A.M.R., Sheerman, S.E., Barker, R.F. and Boulter, D. (1987) A novel mechanism of insect resistance engineered into tobacco. Nature. **330**:160-163.

Hass, G. M., Hermodson, M. A., Ryan, C. A., and Gentry, L. (1982) Primary structures of two low molecular weight proteinase inhibitors from potatoes. *Biochemistry* **21**:752-756.

Ikenaka, T. and Odani, S. (1978) Structure-function relationships of soybean double-headed proteinase inhibitors. *in* Mugnusson, S., Ottesen, M., Foltmann, B., Donø, K., and Neurath, H. eds Regulatory Proteolytic Enzymes and Their Inhibitors. Pergamon Press. Oxford. pp. 207-216.

Johnson, R., Narvaez, J., An, G. and Ryan, C.A. (1990) Expression of proteinase inhibitors I and II in transgenic tobacco plants: Effects on natural defense against *Manduca sexta* larvae. Proc. Natl. Acad. Sci. USA. **86**:9871-9875.

Keil, M., Sanchez-Serrano, J., Schell, J. and Willmitzer, L. (1986) Primary structure of a proteinase inhibitor II gene from potato (Solanum tuberosum). Nucl. Acids Res. **14**:5641-5650.

Klopfenstein, N.B., Shi, N.-., Hall, R.B., McNabb, S.E. and Thornburg, R.W. (1991) A wound-inducible promoter from an herbaceous dicot functions correctly in the woody perennials: Potato proteinase inhibitor II in poplar trees. Can. J. For. Res. (in press).

Kowalski, D. and Laskowski, M., Jr. (1972) Inactivation of enzymatically modified trypsin inhibitors upon chemical modification of the a-amino group in the reactive site. Biochemistry **11**:3451-3455.

Kunitz, M. (1947a) Crystalline soybean trypsin inhibitor. II. General properties. J. Gen. Physiol. **30**:291-310.

Kunitz, M. (1947b) Isolation of a crystalline protein compound of trypsin and of soybean trypsin-inhibitor. J. Gen. Physiol. **30**:311-320.

Kunitz, M. and Northrop, J.H. (1936) Isolation from beef pancreas of crystalline trypsinogen, trypsin, a trypsin inhibitor and an inhibitor-trypsin compound. J. Gen. Physiol. **19**:991-1007.

Kyte, J. and Doolittle, R.F. (1982) A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157**:105-132.

Laskowski, M. Jr. (1986) Proteinase inhibitors of serine proteinase: Mechanism and classification. *in* Friedman, M. ed. Nutritional and Toxicological Significance of Enzyme Inhibitors in Foods. Plenum Press, New York and London. pp. 1-17.

McNabb, H.S., Klopfenstein, N.B., Hanna, R.D., Hall, R.B., Hart, E.R., Heuchelin, S.A., Allen, K.K. and Thornburg, R.W. (1991) A field test of transgenic hybrid poplar trees: Establishment and growth through the second season. *in* Biological monitoring of genetically engineered plants and microbes., MacKenzie, D.R., eds Bethesda, MD. Agricultural Research Institute. pp. 155-159.

Melville, J. C. and Ryan, C.A. (1972) Chymotrypsin inhibitor I from potatoes. J. Biol. Chem. **247**:3445-3451.

Nelson, C. E. and Ryan, C. A. (1980) *In vitro* synthesis of pre-proteins of vacuolar compartmented proteinase inhibitors that accumulate in leaves of wounded tomato plants. Proc. Natl. Acad. Sci. USA. **77**:1975-1979.

Ofelt, C. N., Smith, A. K., and Mills, J.M. (1955) Proteinase of the soybean. Cereal Chem. **32**:53-63.

Park, S. (1991) Characterization of a potato proteinase inhibitor II gene whose expression is not wound-responsive. PhD Dissertation, Iowa State University, Ames.

Pearce, G., Sy, L., Russell, C., Ryan, C. A., and Hass, G.M. (1982) Isolation and characterization from potato tubers of two polypeptide inhibitors of serine proteinases. Arch. Biochem. Biophys. **213**:456-462.

Plunkett, G., Senear, D. F., Zuroske, G., and Ryan, C.A. (1982) Proteinase inhibitors I and II from leaves of wounded tomato plants: Purification and properties. Arch. Biochem. Biophys. **213**:463-472.

Rackis, J. J. and Anderson, R.L. (1964) Isolation of four soybean trypsin inhibitors by DEAE-cellulose chromatography. Biochem. Biophys. Res. Commun. **15**:230-235.

Read, J. W. and Haas, L.W. (1938) Studies on the baking quality of flour as affected by certain enzyme actions. V. Further studies concerning potassium bromate and enzyme activity. Cereal Chem. **15**:59-67.

Richardson, M. (1974) Chymotryptic inhibitor I from potatoes. Biochem. J. **137**:101-112.

Richardson, M. and Cossins, L. (1974) Chymotryptic inhibitor I from potatoes: The amino sequences of subunits B, C, and D. FEBS Lett. **45**:11-13.

Ryan, C.A. (1966) Chymotrypsin inhibitor I from potatoes: Reactivity with mammalian, plant, bacterial, and fungal proteinases. Biochemistry **3**:1592-1596.

Ryan, C.A. (1968a) Synthesis of chymotrypsin inhibitor I protein in potato leaflets induced by detachment. Plant Physiol. **43**:1859-1865.

Ryan, C.A. (1968b) An inducible protein in potato and tomato leaflets. Plant Physiol. **43**:1880-1881.

Ryan, C.A. (1973) Proteolytic enzymes and their inhibitors in plants. Ann. Rev. Plant Physiol. **24**:322-326.

Ryan, C. A. and Ball, A.K. (1962) An inhibitor of chymotrypsin from *Solanum tuberosum* and its behavior toward trypsin. Proc. Natl. Acad. Sci. USA. **48**:1839-1844.

Ryan, C.A. and Hass, G.M. (1980) Structural, evolutionary and nutritional properties of proteinase inhibitors from potatoes. *in* Ory, R. L. ed. Antinutrients and Natural Toxicants in Foods. Foods and Nutrition Press, Westport, CT. pp. 169-185.

Schechter, I. and Berger, A. (1967) On the size of the active site in proteases. I. Papain. Biochem. Biophys. Res. Commun. **27**:157-162.

Thornburg, R.W. (1990) New approaches to pest resistance in trees. AgBiotech News and Information. **2**:845-849.

Thornburg, R.W. (1991) Modes of expression of a wound-inducible gene in field trials of transgenic plants. *in* Biological monitoring of genetically engineered plants and microbes. MacKenzie, D.R., eds Agricultural Research Institute, Bethesda MD. pp.147-154.

Thornburg, R.W., An, G., Cleveland, T.E., Johnson, R. and Ryan, C.A. (1987) Wound-inducible expression of a potato inhibitor II-chloramphenicol acetyltransferase gene fusion in transgenic tobacco plants. Proc. Natl. Acad. Sci. USA. **84**:744-748.

Thornburg, R.W., Kernan, A. and Molin, L. (1990) CAT protein is expressed in transgenic tobacco in field tests following attack by insects. Plant Physiol. **92**:500-505.

von Heijne, G. (1984) How signal sequences maintain cleavage specificity. J. Mol. Biol. **173**:243-251.

Walker-Simmons, M. and Ryan, C.A. (1977) Immunological identification of proteinase inhibitor I and II in isolated tomato leaf vacuoles. Plant Physiol. **60**:61-63.

Weiss, E. A., Gilmartin, G. M. and Nevins, J. (1991) Poly(A) site efficiency reflects the stability of complex formation involving the downstream element. EMBO J. **10**:215-219.

GENETIC MAPPING OF *Populus*

H.D. Bradshaw, Jr. & R.F. Stettler
Department of Biochemistry and College of Forest Resources
University of Washington, Seattle

In trees, most phenotypic traits of commercial importance are the products of gene action at several or many loci. Detailed genetic maps, now available for a few herbaceous plants, have proven useful as tools for counting and identifying genes that are responsible for quantitatively inherited traits. Application of high-density linkage mapping to problems of quantitative inheritance in trees has been delayed because of the lack of suitable pedigrees with sufficient segregating genetic and phenotypic variation. To address this problem, we have produced and begun to map a three-generation inbred pedigree founded by hybridization between a female *Populus trichocarpa* Torr. & Gray (T) and a male *P. deltoides* Bart. (D). The F_1 (TxD) contains 23 hybrid offspring. Two F_1 hybrids were crossed to produce the F_2 (TDxTD), and each F_1 was backcrossed to one of the founding parents to produce the two possible backcrosses (TDxD; TxTD). Among the 612 advanced-generation offspring, there is striking variation in phenology, form, and growth. Genetic variation is assessed and linkage analysis performed using DNA markers, primarily restriction fragment length polymorphisms (RFLPs). Linkage blocks containing several markers have been assembled, and the search for correlations between RFLP alleles and quantitative traits related to productivity is underway.

Peripheral to the mapping of quantitative trait loci we have found that some TxD hybrids are not diploid, but triploid or aneuploid. This was first revealed by inheritance of *both* maternal RFLP alleles at some loci in some individual F_1 hybrids. Tri/aneuploidy in the hybrids has been confirmed by biochemical analysis of nuclear DNA content and by chromosome counts. As is typical of triploid aspen, triploid TxD hybrids have larger leaf epidermal cells and larger leaves than diploids. Other phenotypic traits, such as leaf shape, abaxial leaf color, and stomatal physiology, are skewed in the direction of the female "T" parent. At least 10 different female *P. trichocarpa* clones in our breeding program have produced one or more tri/aneuploid TxD hybrids as judged by abaxial leaf color. The ramifications of tri/aneuploidy for tree growth, wood quality, and fertility are being investigated.

GENERAL SESSION II

SEED ORCHARD MANAGEMENT

SEED ORCHARD MANAGEMENT --
SUCCESSES, PROBLEMS AND CHALLENGES

D. L. Bramlett[1]

Abstract.--Managers of southern pine seed orchards have
enjoyed major successes and encountered problems in the 40 years
since the inception of the tree improvement program. They now
face challenges for the future. The successes include the
establishment of more than 10,000 acres of southern pine orchards,
the development of protection procedures for cone and seed crops,
the development of cultural practices to increase the production
of flower crops and to maintain tree health, the development of
indoor breeding of orchards, and the establishment of advanced
generation orchards.

Problems include pollen contamination in orchards,
environmental hazards and regulations associated with pest
management, soil compaction and management, and clonal and annual
variation in flower production.

Several challenges must be met if the quality and quantity of
genetically improved seed are to continue to increase. We must
devise alternative pest management procedures, develop an
effective operational supplemental mass pollination procedure, and
improve our methods of collecting, extracting, and storing pollen
to insure high pollen viability and pollen vigor. We must
increase our understanding of the basic reproductive biology of
the southern pines, and must develop methods of increasing
productivity of male and female flowers in young orchards.

INTRODUCTION

Until the southern pine tree improvement programs, seed for nurseries were
collected from trees in natural stands, on logging sites, in fence-rows or
along roads. Pine seed orchards were established under the tree improvement
program. In these orchards, grafts or seedlings of selected phenotypes are
planted in large blocks for the single purpose of producing genetically
improved seeds to supply nurseries. (Zobel et al 1958). There have been a lot
of changes in the management of seed orchards since the southern pine seed
orchards were established in the early 1950's. In this paper I will discuss
some of the successes, problems and challenges of seed orchard management.

---

[1]/Plant Physiologist, USDA Forest Service, Southeastern Forest Experiment
Station, Rt. 1, Box 182A, Dry Branch, Georgia.

There is no question that the seed orchard program has been a big success and it is now taken for granted that the reforestation program in the South has an adequate supply of genetically improved seed. It has not always been this way. Many people have contributed to the overall success of the program. Five major successes have influenced the supply of seed for the South. These are:

- Establishment of over 10,000 acres of southern pine seed orchards

- Protection procedures that greatly increased cone and seed yields

- Development of cultural practices that increase flower production and tree health

- Development of technology for greenhouse breeding orchards

- Establishment of advanced generation orchards on improved sites

Establishment of 10,000 Acres

Certainly, the establishment of over 10,000 acres of southern pine seed orchards must be considered one of the tree improvement program's major successes. The fact that more than 1 billion southern pine seedlings are grown from genetically improved seed each year is testimony to the success of these seed orchards. Those who established these orchards had to overcome many problems. In the early years, grafting and planting required a major effort that involved many individuals. Then seed orchard maintenance, tree breeding, progeny testing, roguing, cone collection, and other procedures had to be carried out. These demanding tasks were completed by a group of dedicated individuals who had great confidence in the success of the program, and most companies and organizations had established first generation orchards by the 1960's.

Protection

As soon as the newly established seed orchards began to produce flowers, cones, and seeds, it became obvious that insect pests were going to be a major concern. The early work of Ed Merkle, Harry Yates, Gary DeBarr, Scott Cameron, Larry Barber, and others identified a host of insects that attacked cones and seeds. Although identification was important, protection procedures were desperately needed. The workers who devised the first protection procedures used modified agricultural or fruit crop sprayers and whatever chemicals were available. Later, intensive field tests identified specific pesticides for use in southern pine seed orchards. Gary DeBarr, Hans Van Buijtenen, and the tree improvement cooperatives deserve a lot of credit for carrying out the many large-scale tests that were required. Larry Barber made a significant contribution when he developed aerial application procedures that insure that pesticide coverage is correct.

Protection of seed orchards was unquestionably a major contribution to the production of seed from southern pine seed orchards. The seed crop produced by pines in unprotected natural stands is less than 10 percent as large as the biologically potential seed crop. Performance of unprotected pines in planted orchards is this bad or even worse. Godbee et al. (1977) reported that an unprotected slash pine seed orchard yielded only 6 percent of the potential seed crop. However, as the level of protection increases the production of seeds increases dramatically. For example, a 50-acre loblolly pine seed orchard with an average of 400 flowers per ramet and 50 trees per acre at age 14 could produce 10,000 pounds of seed (Bramlett 1987). At $80.00 per pound, the 10,000-pound crop would be worth $800,000. With no protection the actual harvest would be only 1,000 pounds, or enough seed to plant approximately 10,000 acres. Even with maximum protection the yield does not equal the biological potential, but we estimate that up to 54 percent of the potential seed can become plantable seedlings. This means that the same 50-acre orchard would yield seed worth $432,000, or enough seed to plant 54,000 acres of regeneration. Also, the protected orchard would produce as many seed on 10 acres as an unprotected orchard would produce on 50 acres.

Cultural Practices

Until the southern pine tree improvement program, no one had attempted to grow pine seeds in an orchard environment. Thus, it was necessary to identify cultural practices that would increase seed production and protect the health of orchard trees. Previous studies in natural stands led researchers to test fertilization, irrigation, and other cultural practices in seed orchards. Ron Schmidtling (1974) and J. B. Jett (1987) were especially diligent in identifying the nutritional requirements for good flower production in seed orchards. Their work indicated that additional nitrogen was beneficial even when the trees were receiving a balanced fertilizer application for tree health and vigor. To my knowledge, micronutrients have not been demonstrated as limiting in outdoor seed orchards in the SE United States.

Subsoiling has also been effective in promoting flower production in seed orchards. Subsoiling improves soil aeration by shattering compacted soils and by pruning lateral roots that are growing into the traffic area. The net effect is increased flower production. Subsoiling may have to be repeated periodically and in varying patterns (Jett 1987).

Irrigation has not consistently increased flower production. However, irrigation during the orchard establishment phase (ages 1-5) can promote the development of large vigorous crowns that have the capacity to support large flower and cone crops after irrigation has been discontinued.

Indoor Breeding Orchards

During the first generation of orchard establishment, most breeding of progeny to evaluate phenotypic selections was done in the production orchards. As trees produced flowers and pollen, they were crossed to provide seed for large-scale progeny tests. The procedure, however, was dependent on the initiation of adequate flowering under orchard conditions and subject to seed and cone losses from insects, diseases, and weather. To reduce the generation interval, Mike Greenwood and Claude O'Gwynn developed an innovative indoor

breeding procedure. In this procedure, out-of-phase dormancy is induced by growing potted pines under moderate water stress in a greenhouse environment. These potted grafts of selected phenotypes are held for extended periods in a flower inductive state and initiate both male and female flowers earlier than is possible in outdoor breeding orchards or operational seed orchards. Viability of pollen from indoor grafts has also been adequate to complete necessary crosses for progeny testing. This very innovative approach has greatly accelerated the establishment and roguing of advanced generation orchards by reducing the pine generation interval. Future seed orchards will undoubtedly combine early genetic evaluation and indoor breeding technology to continue rapid progress in southern pine tree improvement.

Second Generation Orchards

The establishment of second generation orchards was an important achievement. This is because of the great improvement in the site selection, orchard management, and protection of the second generation orchards. First generation orchards were a learning experience for most organizations. The second generation orchards, however, represent the second cycle of recurrent selection and potentially a more valuable seed crop. Second generation orchard sites were established in geographic areas known for good flower and cone production. Some organizations located second generation orchards on productive sites far from their operational holdings. Careful attention to soil properties was part of the location selection process: many first generation orchards had been established on soils that were to wet or were otherwise unsuitable. In addition, grafting success and tree uniformity have been greater in second generation orchards, which have also had wider spacing, earlier roguing, and better maintenance and protection. The combination of these conditions has led to earlier cone and seed production. It is unlikely that as much improvement will be made in succeeding generations of orchards, but further fine tuning can be expected for to produce additional gains for several more breeding cycles.

PROBLEMS

Despite the overall success of the tree improvement program and the high level of production in seed orchards, problems arose that reduced the quantity and genetic quality of seed. The four major problems have been:

· Contamination by non-orchard pollen

· Environmental hazards and regulations associated pest management

· Soil compaction and management

· Clonal and annual variation in flower production

Contamination of Orchard Pollen

Right from the start of the tree improvement program, it has been recognized that pollen contamination is a problem for seed orchards. Isolation or dilution zones around the orchards were expected to reduce the inflow of

outside pollen. The theory was that released pollen settles rather rapidly and that most of the pollen in an orchard surrounded by an isolation zone would be from the orchard's trees. Unfortunately, pollen is so buoyant that it is easily lifted in the atmosphere and may be transported for long distances. Lanner (1966) described the atmospheric conditions that could lead to long distance transport of pollen. In the southeastern US, pollen is first dispersed in the warmer southern latitudes. This pollen may be deposited locally but may also be transported by the prevailing wind. When the wind reaching an orchard is from a warmer climate, pollen is deposited in the orchard before the orchard pollen is released. Thus, as soon as female flowers are receptive in a given orchard, outside pollen will begin to contaminate the orchard selections. The problem of contamination increases as more and more local pollen is shed, and by the time of peak orchard pollen shedding, the whole atmoshpere contains very high concentrations of pine pollen. Estimates of pollen contamination for most seed orchards are in the 40-60 percent range. Furthermore, the consequences of pollen contamination will increase with each generation of improvement as the genetic value of the seed increases. Solutions will not be simple. The Australians have the good fortune to grow Pinus caribaea seed orchards far from the natural range of the species. It is also possible to isolate orchard trees reproductivity by altering or taking advantage of patterns of flowering phenology. Phenological isolation has been achieved for Douglas-fir in the Pacific Northwest and Canada El-Kassaby 1989). When the genetic value of orchard seed becomes high enough, other methods of avoiding pollen contamination may be justified. Orchards might be planted in clonal rows or clonal blocks, and supplemental or controlled pollinations and physical barriers to pollen might be employed to reduce the impact of unwanted pollen on the best genetic selections.

Environmental Hazards and Pesticide Regulations

The last decade has seen an increased awareness of environmental issues. These environmental concerns are important to each of us, yet growing pine seeds in a seed orchard without chemical protection would be extremely difficult. Bill Lowe and a SFTIC subcommittee are working to maintain the list of pesticides available for use in seed orchards, but there is a good possibility that the registration pesticides in current use could be withdrawn.

One of the problems is that the combined seed orchard acreage is only a very small market for a pesticide, compared to agricultural or horticultural use. Thus, we cannot expect that new compounds will be developed specifically for use in pine seed orchards. We will have to depend on existing pesticides. Fortunately, seed orchards have recently been classified as terrestrial non-food sites rather than forested areas. This means that horticultural or nursery products may be more readily available for orchard use.

The other problem is the public perception of aerial application of pesticides. Regardless of the low risk to human health or environmental safety of a pesticide, orchard neighbors and the public in general are opposed to aerial application. Even though aerial application may increase the efficacy of a pesticide, this technique may become unacceptable from a public opinion viewpoint.

Soil Compaction

Although the actual quantitative effect of soil compaction on seed production is not known, the continued use of aerial lift trucks, tractors, and other vehicle equipment is certainly detrimental to the physical properties of orchard soil. This problem can be partially alleviated by subsoiling as suggested by Jett (1987). Even so, orchard sites suffer from continued traffic over an extended period of time. Equipment that exerts less pressure on the soil surface is certainly more desirable as is minimization of traffic during periods of high soil moisture. Also orchard sites should be carefully selected for soils that are not easily damaged, and that have good surface and subsurface water drainage.

Clonal and Annual Variation

One of the problems of seed orchard management has always been the large amount of clonal and annual variation in fecundity. Clonal variation is a recognized fact of orchard management, yet its causes are little understood (Schmidtling 1983). Although it sometimes seems that the best genetic clones are the worst seed producers, there is little hard evidence that outstanding vegetative growth is negatively correlated with reproduction growth. The ideal seed orchard clone would produce abundant flowers and cones under seed orchard conditions yet give rise to progeny that would demonstrate only vegetative growth when grown in forest plantations.

Annual variation is also largely a mystery. The current theory is that good seed crops are favored when the inductive period (July and August) is relatively dry after a moist spring. The idea is that good shoot growth in the spring develops strong branches and that mild water stress causes buds to remain in the resting stage during the flower initiation period. Unfortunately, flowering is not strongly related to measurable weather variables such as monthly rainfall or temperature. Local conditions, microsite variability, tree age, and crown characteristics all influence the flower initiation process. It also appears that regional effects are important, because seed production is good in orchards throughout the South in some years and generally poor in other years.

There also appears to be an interaction between annual seed production and clonal production. In general, clones are consistently good or poor producers, but some clones change in rank between years. It would appear that a large cone crop would deplete a tree's carbohydrate reserves and that fewer cones would be initiated the following year. However, since crops of pine seeds require two years to develop, depletion of carbohydrates would favor production of cones every third years.

I see 5 challenges that must be met if southern pine seed orchards are to continue to yield large quantities of high quality seed. These are:

- Development of alternative pest management procedures

- Development of effective operational supplemental mass pollination procedures

- Development of better understanding of basic reproductive biology of southern pines

- Development of methods that will increase male and female flower production in young orchards

- Development of improved pollen collection, extraction, and storage methods that will insure high viability and vigor

Alternative Pest Management Procedures

Protection is vital to maintenance of high levels of seed production. DeBarr (1981) discusses different approaches to protection in seed orchards. Certainly safer and more effective chemical pesticides would be useful if they meet the increasingly stringent environmental requirements. Biological control offers exciting possibilities, but it is most unlikely that biological control will be as effective as the chemical methods now employed. Lack of a reliable arsenal of pesticides or effective biological control methods would drastically change the way that orchards are managed. Much greater orchard acreages would be required, and this could force organizations to look for other methods of producing the seedlings required for regeneration or for other locations in which to establish orchards.

Development of Supplemental Mass Pollination

An effective supplemental mass pollination (SMP) procedure would greatly reduce the detrimental effects of pollen contamination. Although SMP is a very simple concept, the actual yields of SMP seed on an operational level have been disappointing. The actual application of pollen to receptive female flowers has been relatively easy. Pollen is applied using a funnel on the end of a wand with an air delivery system (Bridgwater et al. 1987). The funnel is placed over the flower cluster and approximately 1 cc of pollen is dispersed over the flowers. As 1 cc of pollen has about 10,000,000 pollen grains, the probability is high that each ovule will have one or more SMP pollen grains attached to the micropylar arms. The problem is that there is a very narrow window of opportunity for applying the pollen to a particular flower. SMP appears to be most effective at a flower stage of late 4 (4.5-5.0). However, wind-borne pollen is being dispersed during the same time period and may also be found on the micropyle. If SMP occurs too soon, a lot of wind pollen will accumulate after SMP and will be transported to the pollen chamber in the pollination droplet. If SMP is too late (after the pollination droplet has emerged) it is almost certain that the SMP pollen will not be the male parent.

But even when the SMP is timed perfectly, the SMP pollen will have to compete with wind-borne pollen. Unfortunately, pollen tube growth of SMP pollen may be less vigorous than pollen tube growth of wind-borne pollen. Thus, the wind-borne pollen may be outnumbered by SMP pollen in the pine ovule, and yet be more competitive in completing fertilization and parenting the offspring from the seed orchard. The phenology of the female parent also contributes to the success of SMP. Clones with early female receptivity appear to be most adapted to SMP. Rates of SMP success for clones that flower late or in mid-season may be very low.

It will be a challenge to find methods for isolating receptive female flowers from wind-borne pollen long enough to complete SMP. Protective bagging, precise timing, or location of orchards in different geographic areas may be possibilities, but considerable work is needed to make SMP an operational success.

Reproductive Biology of Southern Pines

The reproductive biology of gymnosperms intrigued late 19th and early 20th century botanists, who conducted several outstanding studies of the pine life cycle. After this early work, however, little new information was added until recently, when the economic value of genetically improved seed justified additional research on the reproductive process. John Owens and his coworkers in Canada have produced outstanding descriptions of reproductive processes of western conifers, but current research on loblolly and other pines is sadly lacking. The time of fertilization of loblolly was observed by March et al. (1989). The life cycle process is apparently similar for all pines, but just how these processes are affected by orchard management practices is not known. For example, it is possible that levels of orchard fertilization change the number of archegonia formed per ovule. If heavy applications of fertilizer had this effect, the potential number of fertilization per ovule would be increased and the treated orchards "buffered" against relatively high levels of self pollination. This buffering could result in a reevaluation of orchard planting design or the use of clonal rows or clonal blocks to increase the efficiency of orchard management.

Additional work is also needed on pollen phenology and pollen dispersal within the orchard. Research by Boyer (1981) demonstrated that pine phenology was related to a cumulative heat sum. Other researchers have shown that dispersal patterns vary among seed orchard clones (Askew 1986, Blush et al. 1992, Bramlett and Bridgwater 1989).

Better understanding of pine phenology and pollen dispersal patterns will enable managers to increase the genetic quality of seed orchard seed through improved orchard design and improved pollination procedures.

Increase in Male and Female Flowering

Second generation orchards are producing clones and seeds sooner than did the original orchards. It would be better if trees began producing even earlier, because the most advanced selections will be in the youngest orchards and these trees will produce the most valuable seed. Much of the acceleration in production has resulted from better site selection and orchard management.

I do not think we have pushed the reproductive capacity of the trees far enough.

Recent results of stem girdling and $GA_{4/7}$ applications in a 10 years old loblolly pine seed orchard have been encouraging (Wheeler and Bramlett 1991). The combination of girdling and GA increased female flowering by approximately 300 percent. The treatment was effective for all test clones but the response was related to be flowering level of untreated controls.

Additional research is needed to determine the responses of young pines to flower stimulation treatments, the optimum timing of flower stimulation, and the rates of GA that produce the maximum response. Then the treatments must be incorporated into orchard management strategy.

Improved Pollen Processing and Storage

Extracted orchard pollen is used in SMP and is used in testing the genetic potential of selected phenotypes. When the vigor of orchard pollen is reduced by processing or storage, the background wind-borne pollen has an advantage. We now have evidence that the pollen vigor is reduced in extracted pollen stored in a refrigerated desiccator for more than 1 year (Bramlett and Matthews in press). Just how much pollen vigor is reduced by collection, extractions, and freezer storage is not known. We must evaluate the whole process of pollen processing and then develop a protocol that minimizes the reduction in pollen vigor. It also may be that pollen from some clones is more competitive than others in the fertilization process. Pollen competition could be an important factor in the success of SMP.

CONCLUSIONS

Without a doubt, the southern forest tree improvement program can be proud of its success in seed producing from pine seed orchards. The establishment and protection of the first generation orchards were major achievements. Furthermore, the cultural management practices developed have been transferred to second generation orchards. And second generation orchards have been very productive because of excellent site selection, good management, and high levels of protection.

The problems of contamination by outside pollen continues to plague the orchard managers, and its negative impact will increase with advancing generations of selections. Difficulties associated with pest management may well be the most serious threat to continued seed orchard productivity. The long-term effect of soil compaction is not known. If we understood the causes of annual and clonal variation in flower production we would be better able to manage for adequate annual production of seed.

Seed orchard managers will face many challenges over the next decade and in the 21st century. Certainly alternative pest management procedures must be developed. In addition, an effective operational SMP procedure is needed if we are to capture the high genetic gain of specific full-sib crosses. If we are to make further increases in seed production we need to have a better understanding of the reproductive process in pines and have ways to maintain high viability and vigor of processed and stored pollen. Finally, treatments

that consistently increase female flower and pollen production in pines must be developed to provide management expanded opportunities to increase seed production.

The key to successful seed orchard management will be to intensively manage fewer ramets on smaller acreages. If production of flower and cone crops can be stimulated and high levels of protection maintained, the size of seed orchards could be reduced by 50 percent or more. If vegetative propogation becomes economically feasible the size of seed orchards may be further reduced. Even so, the seed production process will continue to be vital to the tree improvement program and will be necessary to provide the genetically improved seedlings for reforestation of the southern forests.

LITERATURE CITED

Askew, G.R. 1986. Implications of non-synchronous flowering in clonal conifer seed orchards. P. 182-191 in Proc. IUFRO Conf. Breeding Theory, Progeny Testing, and Seed Orchards. N.C. State Univ., Raleigh, NC.

Blush, T.D., D.L. Bramlett, and Y.A. EL-Kassaby. 1992. Reproductive phenology of conifer seed orchards. in Pollen Management Handbook Vol II. USDA For. Serv. Agriculture Handbook (In Press).

Boyer, W.D. 1981. Pollen production and dispersal as affected by seasonal temperature and rainfall patterns. P. 2-9 in Pollen Management Handbook, E.C. Franklin (ed.). USDA For. Serv. Agriculture Handbook 587.

Bramlett, D.L., and F.R. Matthews. 1992. Pollen storage for loblolly pine. Southern Jour. Appl. Forestry (In Press).

Bramlett, D.L. 1987. Protection of pine seed orchards in the southeastern United States. Forest Ecol. Manag. 19:199-208.

Bramlett, D.L., and F.E. Bridgwater. 1989. Pollen development classification system for loblolly pine. P. 116-121. in Proc. 20th Southern For. Tree Impr. Conf. Charleston, SC.

Bridgwater, F.E., D.L. Bramlett, and F.R. Matthews. 1987. Supplemental mass pollination is feasible on an operational scale. P. 216-222 in Proc 19th Southern For. Tree Imp. Conf. College Station, TX.

DeBarr, G.L. 1981. Prospects for integrated pest management in southern pine seed orchards. P. 343-354 in Proc. 16th Southern For. Tree Imp. Conf. Blacksburg, VA.

El-Kassaby, Y.A. 1989. Genetics of Douglas-fir seed orchards: expectations and realities. P. 87-109 in Proc. 20th Southern For. Tree Imp. Conf. Charleston, SC.

Godbee, J.F., T.S. Price, and D.L. Bramlett. 1977. Effect of an insecticide spray program on slash pine seed orchard efficiency. P. 108-115. in Proc. 14th Southern For. Tree Imp. Conf., Gainesville, FL.

Jett, J.B. 1987. Reaching full production: a review of seed orchard management in the southeastern United States. P. 34-58 in Proc. IUFRO conf. Breeding Theory, Progeny Testing, and Seed Orchards. N.C. State University, Raleigh, N.C.

Lanner, R.M. 1966. Needed: a new approach to the study of pollen dispersion. Silvae Genetica 15: 50-52.

March, R.A., W.V. Dashek, D.L. Bramlett, and J.E. Mayfield. 1989. Time of fertilization in Pinus taeda L. (Loblolly pine). Biodeterioration Research 2: 583-595.

Schmidtling, R.C. 1974. Fruitfulness in conifers: nitrogen, carbohydrate, and genetic control. P. 148-164. in Proc. of 3rd North American Forest Biol. Workshop, College of Forestry and Natural Resources, Colorado State Univ., Fort Collins, CO.

Schmidtling, R.C. 1983. Genetic variation in fruitfulness in a loblolly pine (Pinus taeda L.) seed orchard. Silvae Genetica 32:76-80.

Wheeler, N.C. and D.L. Bramlett. 1991. Flower stimulation treatments in a loblolly pine seed orchard. So. Jour. Appl. Forestry 15:44-50.

Zobel, B.J., J. Barber, C.L. Brown, and T.O. Perry. 1958. Seed orchards-- their concept and management. J. Forestry 56:815-825.

STAGES OF FLOWER DEVELOPMENT AND CONTROLLED-POLLINATED
SEED YIELDS FOR AMERICAN SYCAMORE

S. B. Land, Jr.[1]

Abstract.--Six stages of bud and flower development of American sycamore are described and used to determine optimum controlled-pollination procedures. Flowers on trees from southern origins developed about three days ahead of flowers on trees from more northerly origins in Mississippi when all were grown at the same site, but flowers from all origins were susceptible to frost kill during stages 4 and 5. Yields of seed balls and full-seed percentages indicated that: (1) female flowers should be bagged by mid stage 4 and control-pollinated at mid to late stage 5, (2) bagging was effective in preventing outside pollination and should not be removed until flowers reach mid stage 6, and (3) up to 24 days of bagging had no detrimental effect on flower survival or seed yield. Most mortality of bagged flowers occurred during the three weeks after bag removal, and this was also when most growth of the seed ball occurred. Male flowers should be collected during early to mid stage 5, and they will yield approximately 0.01 ml of pollen per flower ball.

Keywords: Platanus occidentalis, flowering, controlled-pollination.

INTRODUCTION

Controlled pollinations among selected trees are often required for basic genetics studies and for multiple-generation improvement programs. Effective pollinations are dependent on: (1) recognition of the developmental stages of flowers and (2) knowledge of when to isolate female (pistillate) flowers, when to collect male (staminate) flowers, when to apply pollen to the female flowers, and when to remove isolation bags. Such information has been illustrated by Bramlett and O'Gwynn (1980) for southern pine flowers. Publications on flowering and fruiting of American sycamore (Platanus occidentalis L.) (Bonner 1974, Wells and Schmidtling 1990) do not provide sufficient detail for controlled pollination purposes. The objectives of this paper are to: (1) describe morphological stages of sycamore flower development and (2) identify optimum stages for bagging, pollen collection, pollination, and release.

METHODS

Bud appearance and degree of flower emergence were categorized into developmental stages and recorded at approximately two-day intervals from March

20 to April 10, 1987, and from March 23 to April 28, 1988, on 18 clones in a grafted sycamore orchard in east-central Mississippi (33°17'N, 88°54'W). Six clones had origins in southwest Mississippi and central Louisiana, six had origins in central Mississippi and southern Arkansas, and six had origins in northern Mississippi and central Arkansas.

In 1988, single-pair matings with reciprocal crosses were made within each of the three origin groups. Self-pollinations were also made when there were sufficient flowers. Female flowers were bagged in sausage casing (14-cm diameter; from TEE-PAK, Inc., Oakbrook, IL) during April 2-8, and stage of female flower development was recorded at time of bagging. Male flower balls were collected during April 2-13, and stage of male flower development was recorded at time of collection. Male flowers were dried in sausage-casing packages under warm lights and then shaken through a fine wire screen into vials. The cotton-stoppered vials were stored in a dessicator containing "Drierite" (anhydrous $CaSO_4$) in a refrigerator to maintain moisture content at one percent and temperature at 2-3°C until used. Controlled pollinations were done with a cyclone pollinator (ERI Machine Shop, Iowa State University, Ames, Iowa), and female flower stages at time of pollination were recorded. When sufficient flowers were available, some bags were not pollinated to provide a check of the effectiveness of bagging. Bags were removed at six to 24 days after pollination, depending on stage of female flowers in bags, and flower stages were recorded.

Seed ball survival, peduncle length, and ball diameter were recorded at monthly intervals from three weeks after bag removal until November 7-9, 1988, when the seed balls were harvested. Open-pollinated seed balls from the clones were collected at the same time. The percent full seeds was determined for each "cross" of each clone (outcrossed, selfed, no pollen, and open-pollinated) from x-ray radiographs. Four 50-seed samples and a 20-seed sample from each of 52 "crosses" were placed on double-sided cellophane tape attached to 20cmx25cm paper cards. These were x-rayed on Kodak Industrex Type M film with an exposure of 25kVP, 3mA, 65-cm film-focus distance for 60 seconds. The 20-seed sample was subsequently used for cutting tests to confirm empty- or full-seed status of radiograph images.

The yield of seed balls harvested (expressed as a percentage of the female flowers pollinated) and the yield of full seeds (expressed as a percentage of the total number of seeds in a seed ball) were averaged across clones for (1) the female flower stages when pollinated, (2) the female flower stages when bagged (when no pollen was put in the bags), and (3) the number of days that the cross-pollinated female flowers were bagged. Means across all clones were also used to illustrate seed ball survival and growth at monthly intervals following removal of bags. SAS procedure REG (SAS Institute, Inc., 1988) was used to determine the overall relationship between number of male flower balls collected and pollen yields for flowers collected at two stages of development. A balanced subset of seven clones having seeds from all four types of "crosses" (open, cross, self, and no pollen) was used in the SAS ANOVA procedure for a Duncan's test of ranked "cross" means.

RESULTS AND DISCUSSION

Six morphological stages of bud and flower development of sycamore were devised for this study and are described in Table 1. The early part of a stage

Table 1. Six stages of bud and flower development in sycamore.

Stage	Description	Time Period in central MS
1	Tight buds; no swelling; vegetative buds indistinguishable from floral buds.	Winter to mid March
2	Swollen buds; bud scales splitting; sap extrusion may occur from buds; vegetative buds indistinguishable from floral buds.	3-4 days in late March
3	Leaf emergence from under bud scales, but "flower ball" (globose head of flowers of one sex) (which may be in same bud) not visible; on some trees the flower ball emerges immediately when scales split, so that stage 3 is skipped.	0-2 days in late March
4	Starts (stage 4-) when flower balls can first be identified as they emerge from bud (female is red, from stigmas, and male is green); females are more frequent on branch tips in top of crown, while males occur throughout crown and are more frequent on branchlets 20-100 cm back from branch tips; by late stage 4 (=stage 4+) the female is a 5-8 mm diameter bright purplish red ball on a 2-3 cm peduncle, and the male is a 1-cm diameter dark green or purplish-green ball on a 2-cm peduncle.	5 days in late March to early April
5	Starts (stage 5-) when the female flower ball enlarges to 1-cm diameter on a 3-4 cm peduncle, color may change to greenish white, and the ball has a soft "waxy" feeling when rubbed between fingers; male flower ball at stage 5- shows some yellow or gold color, and less than 10% of balls on tree have started to open (i.e. pollen released when ball thumped); during stages 5 and 5+ the female flowers retain the soft waxy feeling and the peduncle elongates to 5-6 cm, while 10-50% of the male balls are open during stage 5 and 50-90% are open during stage 5+; stages for males and females may not be the same on the same tree.	Females = 7-10 days in early April; Males = 2-7 days in early April
6	Starts for female flowers (stage 6-) when the ball starts turning brown and is not as waxy feeling; by stage 6 the female is brownish red and dry to touch, with diameter of 10-15 mm; by stage 6+ the stigmas have dried to dead brown and shriveled, so that the background green color of core becomes the dominant color of the young seed ball (diameter of 15-20 mm and peduncle of 5-12 cm); stage 6- for males occurs when 90% of balls have opened and shed pollen and stamens to leave only the flower ball core; by stage 6 for the male flowers, only cores remain on the tree.	Females = last 2 weeks in April; Males = 3rd week in April

is designated by "minus" following the stage number, the late part is indicated by "plus," and the middle part has only the stage number without plus or minus.

In 1987, clones from the southern-origin group were about three days ahead of the other two groups in stage of development (Table 2). There was variation

Table 2. History of bud and flower development for 18 sycamore clones from three latitudinal origins prior to and following a late frost on March 30, 1987, in east central Mississippi.

| Origin & Statistic | Stage of Bud & Flower Development | | | | | | No. Flowers/Tree | |
| | March | | | | April | | Males Collected | Females Counted |
	20	25	27	31	3	6		
Southern (30°45'-31°50'N Latitude) (s.w. MS and e. LA):								
Maximum	3-	4+	5	5+	5+	dead	119	10+
Minimum	2-	4-	4+	5	5	dead	3	2
Average	2.0	4.0	4.6	5.0	5.0	dead		
Central (33°7'-33°40'N Latitude) (c. MS and s. AR):								
Maximum	2+	4	5-	5	5	dead	95	10+
Minimum	1	2	3	4-	4-	dead	none	1
Average	1.7	3.1	3.8	4.5	4.6	dead		
Northern (34°20'-34°35'N Latitude) (n. MS and c. AR):								
Maximum	2	4+	5	5+	5+	dead	209	10+
Minimum	1-	2+	3-	3+	3+	alive	none	none
Average	1.3	3.1	3.7	4.3	4.3	dead		
Overall Avg.	1.7	3.4	4.0	4.6	4.6	dead		

among clones within the same group for rate of development, and there were clonal differences in male and female precociousness of these five-year-old grafts (13-year-old ortets). However, temperatures dropped to 26°F (-3.3°C) on March 30, when all but one of the clones had floral buds in stages 4 or 5. All of these buds were killed, as indicated by arrested bud development and discoloration by April 6. The one clone with stage 3+ buds eventually produced floral buds and seed balls. Apparently, both male and female buds are extremely susceptible to frost kill during stages 4 and 5. Trees with flowers at stage 6 on the nearby Mississippi State University campus did not exhibit frost kill.

In 1988, collection of male flowers before stage 5- yielded very little pollen, while pollen yield from males collected throughout stages 5-, 5, and 5+ was approximately 0.01 ml per ball (Figure 1). Female flowers cross pollinated before stage 4+ produced few seed balls, and those balls had a lower percentage of full seeds than flowers pollinated in stages 4+ to 5+ (Table 3). Pollination bags prevented open pollination of flowers when (1) the bags were placed over the
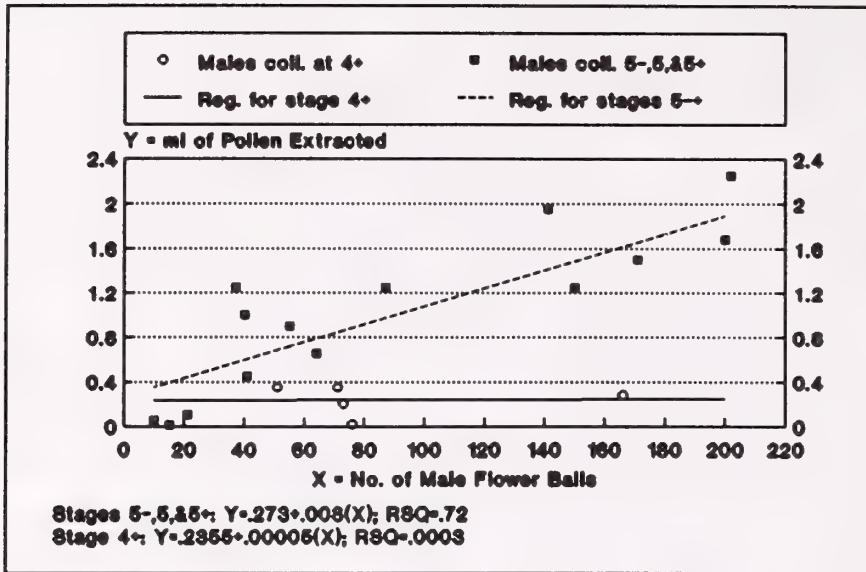
Figure 1. Pollen yields from male flower balls collected at two stages of development.

Table 3. Yields of seed balls and full seeds from female flowers cross-pollinated at different flower stages.

Female Flower Stage When Pollinated	No. of Flower Balls Pollinated	Seed Balls Harvested (% of flowers pollinated)	Full Seeds (% of total seed from ball)
4-	4	25	Not Meas'd.
4	7	14	24
4+	41	41	34
5-	61	51	31
5	15	73	31
5+	34	85	31
6-	2	50	14

female flowers before male flower development reached stage 5- on surrounding trees or (2) late-flowering trees were bagged before female flowers passed stage 4-. This was indicated by full seed yields of only 1-4 percent when flowers were bagged and not pollinated (Table 4), as compared with 23 percent for open-pollinated flowers and 27 percent for cross-pollinated flowers (Table 5). Self-pollinated flowers gave only four percent full seeds. The few full seeds in non-pollinated and selfed seed balls are similar to the results of Beland and Jones (1967), and reconfirm self incompatibility and lack of apomixis in the species. However, flower-ball survival was apparently not dependent on pollination in the present study, as the non-pollinated flowers produced as high a percentage of harvested seed balls as did the cross-pollinated and self-pollinated flower balls. There was no detrimental effect on seed ball survival or full seed yield from keeping cross-pollinated flowers bagged for as long as 24 days (Table 6).

Table 4. Yields of seed balls and full seeds from female flowers bagged at different flower stages and not pollinated.[a]

Female Flower Stage When Bagged	No. of Flower Balls Bagged	Seed Balls Harvested (% of flowers bagged)	Full Seeds (% of total seed from ball)
4-	15	40	0.8
4	5	100	4.0
4+	5	60	1.8
5-	7	71	1.5

[a] All female flower balls were bagged during April 2-4, except for five stage 4- flowers that were bagged on April 7-8 and one stage 4+ flower that was bagged on April 5. Male flower balls reached early shedding stage (5-) on two of 18 clones on April 2 and on six of the 18 clones by April 4.

Table 5. Effects of no pollination, controlled-cross pollination, self-pollination, and open-pollination on yields of seed balls and full seeds from seven sycamore clones.

Type of Pollination	Seed Balls Harvested		Full Seeds (% of total seeds from ball)[a]
	Number	% of Flowers Pollinated	
No Pollen	14	88	2 b
Self	15	94	4 b
Cross	60	71	27 a
Open	147	- -	23 a

[a] Means followed by different letters are significantly different at the 0.05 probability level according to Duncan's Test.

Table 6. Effects of number of days that female flowers were bagged on yields of seed balls and full seeds from controlled cross pollinations.

Number of Days that Flowers Bagged	No. of Female Flower Balls Bagged	Seed Balls Harvested (% of flowers bagged)	Full Seeds (% of total seeds from ball)
10-14	82	60	34
15-19	25	32	29
20-24	54	63	34

Most mortality of bagged female flowers occurred during the three weeks following removal of the bags (Figure 2). Six percent of the flowers died while
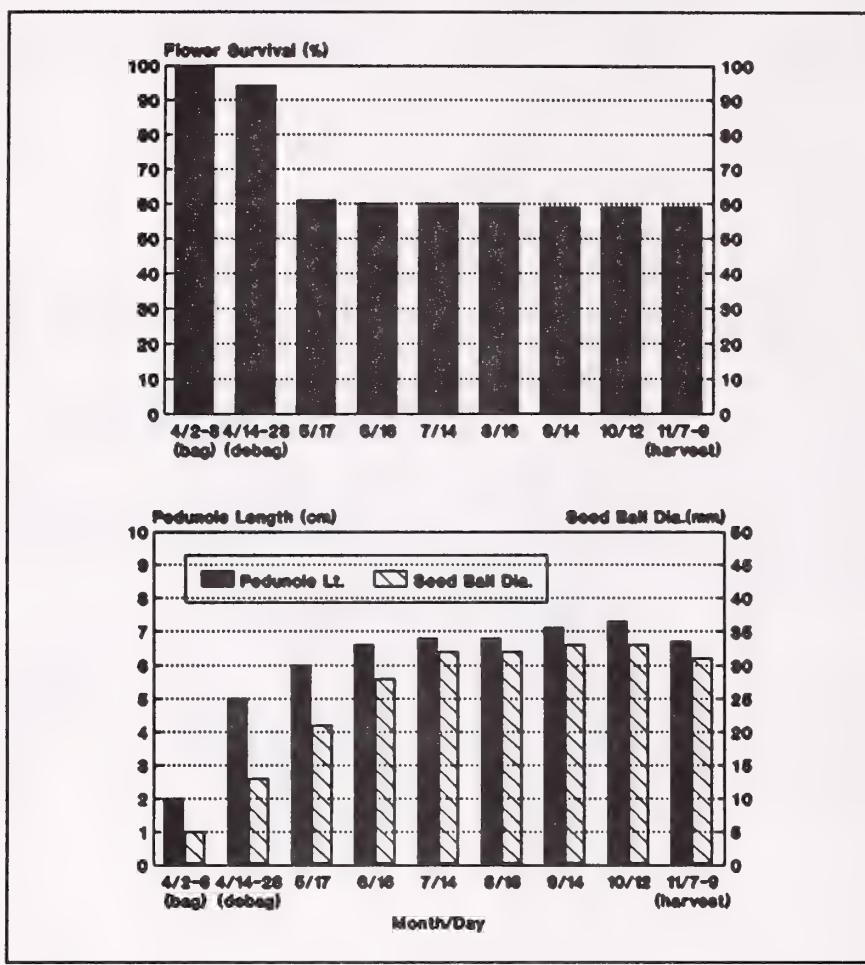
Figure 2. Female flower survival and seed ball development from bagging to seed collection.

in the bags, another 33 percent died during the three weeks after release, and only two percent died after that time. Either some flower balls were dying at time of release, or the flowers were very susceptible to the shock of release. The surviving seed balls had 90 percent of their mature peduncle length and about 70 percent of their mature diameter by three weeks after release from the bags. They continued to grow slowly through mid October, before shrinking slightly in late October as they dried at maturity.

SUMMARY AND CONCLUSIONS

(1)   Six stages of sycamore bud and flower development can be used to determine the best times for bagging, male flower collection, pollination of female flowers, and bag removal.

(2)   Trees from more southerly origins flower earlier, but floral buds in stages 4 and 5 from any origin are susceptible to frost kill.

(3)   Female flowers should be bagged by stage 4 before pollen dispersal. Controlled pollination of these flowers should be done at stages 5 to 5+.

(4)   Male flowers should be collected during stages 5- and 5. Pollen yield during these stages will be approximately 0.01 ml per flower ball.

(5)   Bagging is effective in preventing outside pollination. Bags should not be removed until the female flowers reach stage 6. Up to 24 days of bagging has no detrimental effect on yields of seed balls or seeds.

(6)   Most mortality of bagged flowers occurs during the three weeks after bag removal. The seed balls are about two-thirds of mature size at that time and are attached to peduncles that are nearly the mature length.

LITERATURE CITED

Beland,J.W., and L.Jones. 1967. Self-incompatibility in sycamore. pp. 56-58. In Proc. 9th Southern Conf. on For. Tree Imp. Knoxville, TN.

Bonner,F.T. 1974. Platanus L.: Sycamore. pp. 641-644. In Seeds of Woody Plants in the United States. C. S. Schopmeyer (tech. coord.). Agric. Handb. 450, USDA For. Serv., Washington, DC. 883 p.

Bramlett,D.L., and C.H.O'Gwynn. 1980. Recognizing developmental stages in southern pine flowers: The key to controlled pollination. USDA For. Serv. Gen. Tech. Rpt. SE-18.

SAS Institute, Inc. 1988. SAS/STAT User's Guide: Release 6.03 Edition. SAS Institute, Inc., Cary, NC. 1028 p.

Wells,O.O., and R.C.Schmidtling. 1990. Platanus occidentalis L.: Sycamore. pp. 511-517. In Silvics of North America: 2. Hardwoods. R. M. Bruns and B. H. Honkala (tech. coords.). Agric. Handb. 654, USDA For. Serv., Washington, DC. Vol. 2, 877 p.

FACTORS AFFECTING SEED ORCHARD CROP RATINGS

G.R. Askew and Y.A. El-Kassaby[1]

Abstract. Many factors influence the genetic value of seeds produced in conifer seed orchards. Orchard design factors such as number of genotypes, coancestry among parents, clonal (family) size and spatial arrangement coupled with reproductive phenology and output traits and pollen contamination must be considered when preparing estimates of crop worth. Management practices such as supplemental mass pollination, bloom delay, and selective flower induction as well as selective cone harvesting can be used to adjust the genetic contributions of individual genotypes and to mitigate problems arising from non-orchard pollen. The general genetic value of the population (orchard or seed crop) can be summarized by estimating the average genetic breeding value weighted by the gametic contributions and by the effective population size. Changes in these estimators can be used as indices of the efficacy of management practices and orchard design.

Evaluation of conifer seed orchards on the basis of genetic value is essential for determining the degree of success that has been attained in a breeding program and the performance to be expected from the reforested lands. Seed orchard performance is often rated by the level of annual cone production and the orchards' genetic values are usually listed as average breeding values calculated from progeny test data. Actual genetic value or realized genetic gain potential can be vastly different than progeny test averages and are influenced by factors that are both internal and external to the seed orchard.

An orchard's genetic base is established when trees are chosen and arranged in the orchard. Clonal and seedling orchards have different genetic properties, potentials, and problems.

Clonal and full-sib seedling orchards' breeding values are similar to the clonal value or the mid-point of both full-sib family parent breeding values, respectively. On the other hand, half-sib or wind-pollinated families breeding values represent only 50% of the parents' breeding values.

As indicated earlier, the basic genetic properties of any orchard are defined

by the genetic values of the included parent trees. Tested orchards can be rated from progeny test data in terms of maximum potential given the degree of additive genetic variance among the selections. Test conditions must be representative of operational conditions encountered in the reforestation program. That is, the reliability of the progeny test data to predict future forest potentials lies in the rigor and scope of the testing program. Yield trials are recommended to verify projections based on progeny tests. Orchard seed that is destined for environments excluded from the testing program may perform in unexpected ways. Untested orchards can only be judged from the selection criteria and purely phenotypic information.

Advancing generations of most breeding programs will provide increased information about the genetic value of the trees. Progeny selected from tested, genetically superior families will have parental and grandparental performance documentation. However, information about the genetic composition of any given wind-pollinated seed crop is still incomplete. Gamete contributions to the seed crop are the only reliable estimator of the genetic composition of the seeds and are a necessary ingredient in the calculation of the orchard's genetic value and the seeds' potential (Askew 1989, El-Kassaby and Askew 1991).

Estimating the genetic worth of advanced generation orchards is dependent on the breeding and selection strategy. Advanced orchards established from forward, backward, or forward and backward selections are analogous to tested and untested orchards (i.e. forward selections require further testing).

EXTERNAL FACTORS

Ideally, orchards should be isolated from all trees of the same species to protect the integrity of the pollen cloud. This is rarely the case and isolation or dilution zones are used to minimize the problem of non-orchard pollen con-tamination (Squillace 1967). However, high levels of non-orchard pollen are often found in the seeds despite the use of dilution zones (Squillace and Long 1981, Friedman and Adams 1985, El-Kassaby and Ritland 1986a, Wheeler and Jech 1986). Contaminating pollen will confound the estimates of genetic value and

may eliminate the gain obtained by the breeding program. Stands of trees proximate to the orchard may produce great amounts of pollen and may dominate the pollen cloud. In orchards where reproductive phenology is not synchronized between pollen shedding and flower receptivity (El-Kassaby and Reynolds 1990) or in young orchards, the contamination problem may be overwhelming.

Orchards that are part of a multi-orchard complex may receive contaminating pollen from adjacent orchards of high breeding value. However serious adaptation problems may occur if the orchards represent distinct breeding populations (Friedman and Adams 1985, Wheeler and Jech 1986).

MANAGEMENT PRACTICES

Bloom Delay

Manipulation of reproductive phenology development of seed orchard trees by overhead cooling has proven to be effective in reducing pollen contamination in Douglas-fir seed orchards (El-Kassaby and Ritland 1986a, El-Kassaby and Davidson 1990). Cooling from water spray and evaporation causes floral bud burst to be delayed for as long as two weeks after local pollen release, hence avoiding pollen introgression from outside sources (Silen and Keane 1969, Fashler and Devitt 1980, Fashler and El-Kassaby 1987). Reproductive bud development progresses at a faster pace after the cessation of the cooling treatment and the length of the pollination season is substantially shortened, thus improving panmixis (El-Kassaby et al 1984, Fashler and El-Kassaby 1987). The effectiveness of this method on other coniferous trees has not yet been determined.

Supplemental Mass Pollination

Supplemental mass pollination (SMP) used to enhance the genetic value of orchard seeds by emphasizing specific parents is designed to impact the genetic value of the seed in a positive manner. Effective use of SMP implies the orchard manager has a working knowledge of the reproductive phenology of the orchard.

Timing of flowering and degree of flower production are necessary ingredients for formulating a SMP plan. Application of SMP pollen must be in coordination with the flower receptivity of the target trees and will only improve the genetic value if the supplemented pollen genetic value is significantly higher than the ambient pollen cloud value at the time of application (Askew 1991).

The number of pollen sources to be included in the SMP mix will affect the genetic value of the pollen and the seed produced. Pollen mixes can be constituted to skew the pollen distribution in the toward the highest genetic value parents or they can be constituted by mixing together pollens that are poorly represented in the ambient cloud at the time of orchard receptivity due to asynchrony or poor production. Mixing of the lesser representatives may increase the genetic value of the seed and will increase the effective population size of the seed crop and push the crop closer towards uniform mating probabilities.

Effectiveness of SMP also depends on the viability of the pollen which may decline with age (Webber 1987). Viability tests should be used to allow for modification of the mix ratios and application rates. Viability estimates are also important for calculating the expected average genetic value. The number of applications will also affect the SMP success rate but the economics of SMP must be considered (Askew 1991). Orchards with widely varying phenologies will require more visits to reach the same end as a fairly uniform orchard. SMP success rate should be determined so the genetic value of treated seed crops should reflect the expected improvement.

Insects

Insect predation on large portions of the seed crop has been reported (Belcher and DeBarr 1975). Seed and cone losses may be particularly serious if the predation is not proportional to cone production of the individual clones or families (Askew, et al 1985). SMP work, spatial arrangement, and other management practices to regulate the gamete contribution proportions may be severely impacted if insect predation is non-random or skewed toward a few clones.

Harvesting

Harvesting systems can enhance or dilute the potential genetic gain inherent in the seed crop. Bulk harvests where all cones from all clones are pooled will produce an average valued seed lot. Gamete contributions of individual clones can be calculated from estimates of seed and pollen parent gamete contributions.

Segregating the seed crop by harvesting individual clones and not bulking the seeds allows for calculation of the genetic potential for single mother tree crops in which 1 parent contributes exactly 50% of the gametes. This minimizes the error rate in calculation of the gain (El-Kassaby and Thomson 1990). Matching of seed parents to specific site conditions based on progeny test information can enhance the level of realized gain.

SMP that targets specific clones rather than orchard wide enhancement lends more support to clonal harvests. Genetic gain calculations based on known seed and pollen parents has minimal error.

Contaminating Pollen

The degree of genetic loss inflicted by non-orchard pollen domination of the seed crop is, of course, determined by the genetic value of the contaminating pollen. Orchards located near stands with good phenotypes may cause less genetic loss than those near poor quality, high graded stands. In either case, some form of testing program is warranted to determine the degree of contamination that can be expected. Pollen trapping by sampling the ambient cloud on adhesive covered glass slides can give a fair index of the gross quantity of non-orchard pollen that is entering the orchard (Griffin 1980, Wheeler and Jech 1986). This system cannot establish the genotypes of the pollen or its genetic origin. Isozyme analysis of seeds collected from the surrounding stands can be used to determine the genotypes of the contaminating trees and refine the contamination figures (Smith and Adams 1983, El-Kassaby and Ritland 1986 a&b).

Orchards that are parts of orchard complexes pose another situation. In

these cases, the contaminating pollen may originate from other orchards and be of equal or lesser quality to the desired pollen. Monitoring all orchards in the complex will provide some data about the degree of inter-orchard mating which can be used in the calculation of the gain potential (Friedman and Adams 1985).

Effective Population Size

Monitoring and evaluation of the sources and quantities of pollen relate to a common statistical estimate: effective population size. Small orchards with few potential parents will produce seed lots with narrow genetic bases. Risk factors for widespread use are fairly high relative to larger orchards. However, large orchards with a few parents that dominate the seed crop or the pollen cloud due to fecundity or phenology may reduce the effective population size to that of a small orchard. Contaminating pollen will tend to increase the effective population size by adding more parents to the pool even though the genetic qualities may be suspect. Reproductive phenology studies and genetic gain calculations should include consideration of the effective population size problem and management strategies should be adopted to provide adequate numbers of parent in the gamete pool. These include introduction of pollen (i.e. SMP) from parents that are not in the orchard.

Coancestry

Advanced generation orchards produced from populations generated by controlled mating designs will eventually develop some degree of relatedness among the selections. The degree of relatedness will be determined by the size of the mating design, the selection intensity and the level of avoidance employed during the selection process (Askew and Burrows 1983). Significant levels of coancestry may result in several inbreeding problems in the seed crops (Askew and Burrows 1990, Woods and Heaman 1989). Reduced seed sets have been documented in related matings and inbreeding depression in height growth or disease resistance may be

a problem in some cases. Efforts to subline populations to avoid coancestry may be successful in the short term but eventually the full coancestry problem will be realized as generations continue to advance.

CONCLUSION

Relative values of seed crops are determined by many factors. Some factors are within our control and can be manipulated to attain predetermined goals. Other factors are functions of nature and require monitoring to accommodate their impacts. Estimation of orchard seed values and effects of management practices must utilize all information that is reasonably obtainable if the true value of a breeding program is to be determined.

LITERATURE CITED

Askew, G.R. 1989. Estimation of gamete pool compositions in clonal seed orchards. Silvae Genetica. 37:227-232.

Askew, G.R. 1991. Potential genetic improvement due to supplemental mass pollination management in conifer seed orchards. For. Ecol. Manage. (In press).

Askew, G.R. and P.M. Burrows. 1983. Minimum coancestry selection I. A Pinus taeda population and its simulation. Silvae Genetica 32:125-131.

Askew, G.R. and P.M. Burrows. 1990. Inbreeding prevalence in conifer seed orchards. USDA Pollen Mgt. Hand. II. (in press)

Askew, G.R., R.L. Hedden, and G.L. DeBarr. 1985. Clonal variation in susceptibility to coneworms (Dioryctria spp.) In young loblolly pine seed orchards. For. Sci. 31:794-798.

Belcher, E. and G.L. DeBarr. 1975. Seed orchard survey (SOS): final report and summary of highlights. In: Proc. 13th South. For. Tree Imp. Conf. pp. 145-152.

El-Kassaby, Y.A. and G.R. Askew. 1991. The relation between reproductive phenology and reproductive output in determining the potential gametic pool profile in a Douglas-fir seed orchard. For. Sci. (in press).

El-Kassaby, Y.A. and R. Davidson. 1990. Impact of crop management practices on the seed crop genetic quality in a Douglas-fir seed orchard. Silvae Genetica 39:230-237.

El-Kassaby, Y.A., A.M.K. Fashler and O. Sziklai. 1984. Reproductive phenology and its impact on genetically improved seed production in a Douglas-fir seed orchard. Silvae Genetica 33:120-125.

El-Kassaby, Y.A. and S. Reynolds. 1990. Reproductive phenology, parental balance, and supplemental mass pollination in a Sitka spruce seed orchard. For. Ecol. and Manage. 31:45-54.

El-Kassaby, Y.A. and K. Ritland. 1986a. Low levels of pollen contamination in a Douglas-fir seed orchard as detected by allozyme markers. Silvae Genetica 35:224-229.

El-Kassaby, Y.A. and K. Ritland. 1986b. The relation of outcrossing and contamination to reproductive phenology and supplemental mass pollination in a Douglas-fir seed orchard. Silvae Genetica 35:240-244.

El-Kassaby, Y.A. and A.J. Thomson. 1990. Continued reliance on bulked seed orchard crops: is it reasonable? In: Joint meeting of Western For,. Gen. Assoc. and IUFRO Work. Parties S2-02-05, 06, 12 and 14. Olympia, Wash. (Aug. 1990) 4:56-65.

Fashler, A.M.K. and W.J.B. Devitt. 1980. A practical solution to Douglas-fir seed orchard pollen contamination. For. Chron. 56:237-241.

Fashler, A.M.K. and Y.A. El-Kassaby. 1987. The effect of water spray cooling treatment on reproductive phenology in a Douglas-fir seed orchard. Silvae Genetica 36:245-249.

Friedman, S.T. and W.T. Adams. 1985. Estimation of gene flow into two seed orchards of loblolly pine (Pinus taeda L.). Theor. Appl. Genet. 69:609-615.

Griffin, A.R. 1980. Isolation of a radiata pine seed orchard from external pollen. Aust. For. Res. 10:83-94.

Silen, R.R. and G. Keane. 1969. Cooling a Douglas-fir seed orchard to avoid pollen contamination. USDA For. Serv. Res. Note PNW-101, 10pp.

Smith, D.B. and W.T. Adams. 1983. Measuring pollen contamination in clonal seed orchards with the aid of genetic markers. In: Proc. 17th South. For. Tree Imp. Conf. pp.69-77.

Squillace, A.E. 1962. Effectiveness of 400-foot isolation around a slash pine seed orchard. J. For. 65:823-824.

Squillace, A.E. and E.M. Long. 1981. Proportion of pollen from nonorchard sources. In: Pollen Management Handbook. USDA For. Serv. Agr. Hand. #587, pp.15-19.

Webber, J.E. 1987. Increasing seed yield and genetic efficiency in Douglas-fir seed orchards through pollen management. For. Ecol. Manage. 19:209-218.

Wheeler, N. and K. Jech. 1986. Pollen contamination in a mature Douglas-fir seed orchard. In: Proc. IUFRO Conf. on Breeding Theory, Progeny Testing, and Seed Orchards. Williamsburg, VA. pp. 160-171.

Woods, J.H. and J.C. Heaman. 1989. Effect of different inbreeding levels on filled seed production in Douglas-fir. Can. J. For. Res. 19:54-59.

ROOTSTOCK SCREENING FOR LOBLOLLY PINE SEED ORCHARDS[1]

R.C. Schmidtling
USDA - Forest Service
Gulfport, Mississippi

In two separate but related experiments, seedlings from 20 families were used as rootstocks for grafting seed orchard clones. The rootstocks were half-sib families from orchard clones chosen to represent a wide range of flowering and survival capabilities, based on their performance in a first-generation seed orchard. Ungrafted seedlings from the rootstock families were also planted.

The objectives of the study were to identify rootstock families which would be useful in tree improvement programs and to determine what characteristics, if any, could be used *a priori* to choose families for potential rootstock use.

Rootstock family significantly affected survival, growth, flowering and foliar nutrients of the grafted ramets. Neither survival nor growth of the grafts was related to survival or growth of the orchard clones from which their rootstocks were derived, however. Foliar nutrients of the grafts were not correlated with foliar nutrients of the related seedlings, and this characteristic did not appear to have any utility in predicting performance.

There was a significant tendency for rootstock families derived from good flowering clones to increase the flowering of scions grafted on these rootstocks. Potential causes for this rather curious outcome are discussed.

FLOWERING RESPONSE OF JUVENILE SELECTIONS IN LOBLOLLY PINE

L. C. Burris, C. G. Williams and S. D. Douglass [1]

   Abstract--Four types of loblolly pine scions were grafted
and subjected to female strobilus induction. These scion types
were: age one (1) from nursery-grown seedlings, age three (3S)
from a genetic field test averaging one meter in height, age three
(3L) from a genetic field test averaging 2.4 meters in height and
age eight (8). At 14 and at 26 months after grafting, there was a
positive scion age effect on female strobilus production but none
on male strobilus production. There was more pollen produced on
scion type 3L compared to all other scion types. Grafting and
applying flower induction treatments in the same year could reduce
the breeding interval from five to four years for selections which
are at least three years old. However, this method is not effec-
tive in reducing the breeding interval for early selection methods
which rely on one-year-old selections. All juvenile selections
readily responded to flower induction at 26 months from grafting.

Additional Keywords: Early selection, Pinus taeda L., accelerated
breeding technology

INTRODUCTION

   The flowering response of juvenile selections influences practical use
of early selection schemes for loblolly pine (Pinus taeda L.) in two ways.
Use of juvenile selections can 1) delay flowering response, which offsets the
time savings of early selection, or 2) reduce the flowering response which
raises annual breeding costs, thus reducing gain per unit cost.

   To date, there has been one report on the effect of selection age on
flowering in loblolly pine (Greenwood 1984). Scions were grafted from selec-
tions made in first-generation genetic tests at ages 1, 4, 8 and 12 years.
Flower induction treatments were applied one year after grafting.   In spring
1981, 33 months from grafting, there was a marked reduction between ages 1 and
4 years in male and female flowering response for juvenile selections. Given
refinements in flower induction technology over the past decade, it is not
certain if poor flowering will delay operational use of early selection par-
ticularly if grafting and flower induction, normally separated by one year,
are done in the same year (Figure 1A).

---

[1] Senior Research Technician, Southern Tree Improvement Project Leader and
Project Research Technician, Weyerhaeuser Company, Southern Forestry Research,
P.O. Box 1060, Hot Springs AR 71902.

The effect of juvenile selection on flowering is further clouded by the loose association between chronological age and seedling development (Poethig 1990). Early selection methodology is often based on accelerating seedling growth in a greenhouse (van Buijtenen 1986) or on accelerating seedlings in closely-spaced farm-field genetic tests (i.e. Li et al. manuscript in review; Williams 1987). Accelerated seedling growth may improve flowering response of juvenile selections.

The following study was conducted to address two questions:

1. Can female strobilus induction treatments applied in the same year as grafting be used to reduce the time for breeding juvenile selections?

2. Is height at selection more important than the tree's chronological age in determining flowering response?

METHODS AND MATERIALS

In February 1989, scions were collected for a total of 63 grafts. Four scion types (Table 1) were grafted onto two rootstock types, ages two and three years, using scion from two open-pollinated families from a first-generation North Carolina Coastal Plain seed orchard. Sample sizes of scion age types were nearly balanced: scion type 1 had 17 grafts, scion type 3S and 3L had 15 grafts each and scion type 8 had 16 grafts.

Table 1. Ortet location and site characteristics for each of four scion types.

Code	Scion Type	Selection Age From Seed	Mean Height	Ortet Location	Site Characteristics
1	1 year	10 months	0.20 m	Columbia Co. Arkansas	Weyerhaeuser Nursery
3S	3 year small	34 months	0.99 m	Washington Co. North Carolina	Genetics x silviculture interaction trial. No herbicide applied
3L	3 year large	34 months	2.40 m	Washington Co. North Carolina	Genetics x silviculture interaction trial. Herbicide applied
8	8 year	87 months	4.20 m	Saline Co. Arkansas	Droughty, slow growing site, some competition control

All scions came from the upper one-third of each crown. In May 1989, grafts were repotted from 12-liter pots to 60-liter galvanized metal pots using a mixture of sand, pine bark, peat moss and vermiculite (1:1:1:1 by volume). A slow release fertilizer (Osmocote 18-6-12) was incorporated. The trees had one month to stabilize before starting mid-June female strobilus

induction treatments.

In the first year, 60% of the grafts received gibberellin applications and mild water stress using methods cited by Greenwood (1981). The untreated remainder (40%) served as the control. As described by Greenwood (1981), water stress levels were monitored using a Scholander pressure chamber to measure predawn stress. After reaching stress levels of 125 psi (8.5 atm) trees were watered thoroughly; this continued from mid-June until mid-September. This water stress level of 125 psi is lower than the 150 psi used by Greenwood (1981).

In the second year, all grafts received female strobili induction treatments. Water stress was allowed to reach 150 psi (10.5 atm) before re-watering. A second application of fertilizer was made in April 1990. 70 grams of Osmocote (18-6-12) was added as a top dressing to each tree. Eight applications of 0.01 ml of GA $_{4/7}$ per bud were also applied from mid-June to mid-September using a Cole-Palmer jet pipette model 3202. This was the same application regime used in year 1 flower induction treatment in both years. 1 gram of GA $_{4/7}$ was dissolved per 50 ml of 80% ethanol. All potential flower producing branches were treated as close as possible to the base of the bud.

Grafting scions and applying flower induction treatments within the same year is considered an abbreviated breeding schedule (Figure 1A); flower induction normally follows one year after grafting (Figure 1B). Under the abbreviated schedule, we began female strobili induction at five months after grafting.

(A) Abbreviated breeding schedule   (B) Conventional breeding schedule

Figure 1. Timeline comparison between an abbreviated indoor breeding schedule (A) and conventional indoor breeding schedule (B) as proposed by Greenwood et al. (1986).

STATISTICAL METHODS

The study was blocked on a 2 x 2 rootstock-family combination. Blocks were considered fixed. A linear model with fixed treatment effects was used for analyses of variance:

$$Y_{ijk} = \mu + \beta_i + \tau_j + \beta\tau_{ij} + \epsilon_{ijk}$$

Where μ = overall mean

β_i = effect of block i

τ_j = effect of scion type j

$\beta\tau_{ij}$ = block i by scion type j interaction effect

ϵ_{ijk} = experimental error

A generalized linear model approach was used to adjust for imbalance across scion types for traits based on female and male strobilus clusters. Adjusted means were used to remove any effect of imbalance arising from unequal number of grafts per scion type or from unequal number of strobilus clusters per scion type.

The first-year flower induction treatment was tested as a covariate for all second-year flowering response (26 months after grafting) but demonstrated no statistically important effect on second-year traits. All statistical analyses were aided by PC-SAS version 6.03.

RESULTS

Selection Age Effect at 14 Months on Female Strobilus Production

Accelerated breeding techniques are still effective for loblolly pine; without female induction treatments no scion type would have produced female strobili at 14 months after grafting (Table 2).

Table 2. Response at 14 months after grafting to female strobilus induction treatment applied within six months after grafting across all scion types.

| Trait Description | -- Treatment Response at 14 Mo. -- | |
	Female Strobilus Stimulation Treatment	No Treatment Control
female strobili per graft (count)	0.78	0.04
percentage of flowering grafts (%) (ratio)	34.15% (14/41)	4.50% (1/22)
female strobili per flowering graft (count)	2.30	1.00

Scion types 3S, 3L and 8 produced female strobili at 14 months after grafting (Table 2; Figure 2). The number of female strobili at 14 months was comparable to the 1981 study observed at 33 months after grafting (Figure 2). For example, scion type 8 has 1.2 female strobili per graft which was the same flowering response reported previously for age 8 (Figure 2).

♀ Flowers Per Graft

	1 Year	3 Year Small	3 Year Large	8 Year
14 Months - 1989	0	0.3	0.6	1.2
26 Months - 1990	1.9	4.4	5.4	8.1

Scion Type

33 Months - 1981	Selection Age (years)			
	1	4	8	12
♀ Flowers Per Graft ⟶	0.4	1.0	1.2	2.3
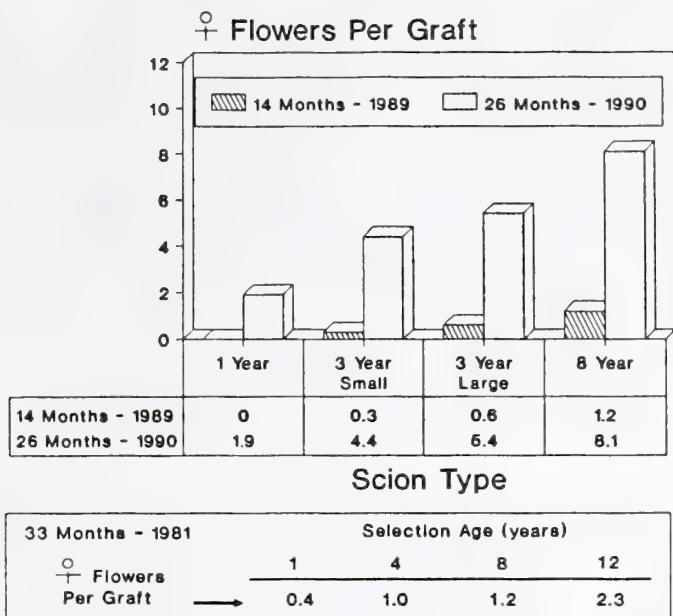
Figure 2. Female flowering response at 14 and at 26 months after grafting. Data are compared with a previous study reported by Greenwood (1984) in which female flowering response was assessed at 33 months after grafting.

However, the same-year graft/flower induction treatment did not induce any female strobili for scion type 1 (Figure 2). In this case, the number of female strobili is less than what was reported for the 1981 study (Figure 2). It is not clear whether this lack of flowering occurred because we used a water stress treatment (125 psi) which was lower than normal.

Selection Age Effect at 26 Months on Female Strobilus Production

Female strobili production increased for all scion types at 26 months after grafting (Figure 2). In absolute terms, scion type 1 in this study had as many female strobili as the 12-year-old scion and four times as many strobili as 1-year-old scion in the 1981 experiment (Figure 2). This was quite unexpected because scion type 1 is younger than the age one scion used in the 1981 study. Age one selections were actually collected from 1.5 to 2.0 seedlings potted from 1-0 nursery stock (Greenwood 1984).

Scion types 3S and 3L had two to three times more female strobili per graft than the 12-year-old scion in the 1981 study and scion type 8 exhibited a four-fold increase compared to 12-year-old selections in the 1981 experiment. Family differences for female strobilus production were negligible (Table 3).

Tree Height Effect on Female Strobilus Production

Height at selection had less influence on female strobilus production than age. The differences between scion types 3S and 3L were statistically significant at the 60% level. This also proved to be the case at 26 months; the differences between scion types 3S and 3L were statistically significant at the 50% level.

Despite a low probability of a true difference due to tree height, scion type 3L did produce more female strobili per graft than scion type 3S at both 14 and 26 months after grafting (Figure 2). The increase in female strobili at 26 months appeared to be due to the number of branch tips bearing strobili although most of these flowering branches bore more singlets (Figure 3).

On a minor point, scion type 3S also had more non-flowering grafts at 14 and 26 months after grafting. At 14 months, 36.4% of the 3S grafts produced female strobili. Of these flowering grafts, there were 1.3 female strobili per graft. For scion type 3L, 44.4% of the grafts produced female strobili. Of these flowering grafts, there were 2.0 female strobili per graft. At 26 months, 73% of the scion type 3S grafts produced female strobili with 6.0 female strobili per flowering grafts. For scion type 3L, 100% of the grafts produced female strobili with a mean of 5.4 female strobili per graft.

Total Branch Tips Bearing Female Flowers

	1 Year	3 Year Small	3 Year Large	8 Year
› 4 per branch tip	0	0	0	2
2-4 per branch tip	7	13	14	36
1 per branch tip	17	34	51	30

Scion Type
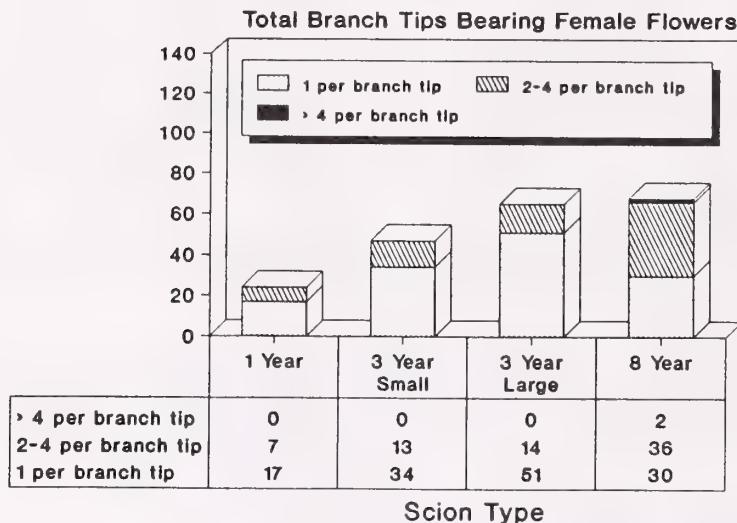
Figure 3. The total branch tips with female strobili are subdivided into three categories: branch tips with a single female strobilus, branch tips with two to four female strobili and branch tips with more than four female strobili. Branch tips bearing female strobili is defined as one terminal and its lateral buds, all of which would be enclosed in one bag for controlled-pollination.

Selection Age Effect on Male Strobilus Production

Pollen catkins did not appear on any grafts 14 months after grafting. This was not unexpected since no pollen induction treatments were applied. Until pollen induction is tested in the same year as grafting, it is uncertain whether pollen will be available in the first year of the abbreviated breeding schedule.

At 26 months, pollen production on all four scion types was adequate for controlled pollinations in a breeding orchard (Bramlett et al. 1985) and there was no detectable scion age effect (Table 4). This is in direct contrast with male strobilus clusters per graft reported in 1981 (Greenwood, 1984; Table 2). There were large differences between open-pollinated families for male strobilus production (Table 3).

115

Tree Height versus Tree Age for Male Strobilus Production

Selection height was more important than selection age for pollen production although the reverse was true for female strobilus production (Table 4; Figure 2). Scion type 3L produced 4.60 male catkin clusters per graft as opposed to 1.35 male catkin clusters per graft for scion type 3S (Table 4). Scion type 3L had fewer male strobili per cluster than any other type yet it produced the most male strobili on a whole-crown basis. The increase was due to height at selection age rather than selection age.

DISCUSSION

Selection Age Effect on Female Strobilus Production

These results suggested that breeding can be started one year sooner for scion types 3S, 3L or 8 by applying flower induction treatments soon after dormant-season grafting. Breeding could be completed in four years rather than in five if pollen is available (i.e. Greenwood et al. 1986). There are added advantage to the abbreviated schedule: the grafts will be smaller at the completion of breeding so that pollination work does not require a ladder. Also, Indoor breeding can be conducted in a smaller greenhouse at a considerable cost savings.

More grafts per selection will be needed if a selection is made in a test with a mean height of one meter. Also, it seemed likely that there will be more non-flowering grafts than if the selections came from a test averaging 2.4 meters in height. Results for scion types 3S, 3L or 8 are applicable to selections from conventional widely spaced tests and perhaps to closely spaced farm-field tests.

Annual breeding costs per graft will also be slightly higher for scion types 3S and 3L compared to age 8. There are more single female strobili per branch tip (singlets) so more isolation bags will be required to obtain the same amount of sound seed. A singlet is less desirable for controlled-pollination than two to four female strobili per branch tip because more isolation bags must be applied to obtain the same number of sound seed. Future refinements in accelerated breeding techniques would best be directed toward increasing numbers of female strobili per branch tip rather as opposed to a whole-crown basis.

For age one selections, same-year grafting and flower induction was not effective. There should be adequate female and male strobili to begin breeding on a conventional breeding schedule; an abbreviated breeding schedule is not an option using these flower induction treatments.

Breeding costs are likely to be highest for age one selections. 71% of the flowering branch tips bore a single female strobilus and many isolation bags would be needed per given quantity of sound seed. If age one selections are used, one option might be to reduce the required number of sound seed per cross and increase total number of crosses.

Effect of Height versus Age on Male Strobilus Production

Scion type 3L had more flowering branch tips than 3S and we observed that many of these tips bore both female and male strobili. This may explain why there were fewer male strobili per catkin cluster yet more catkin clusters in total (Table 4). We also observed in the greenhouse that the taller 3L grafts produced more growth cycles (and lateral branches) at a faster rate once grafted. By contrast, scion type 8 tended to produce fewer cycles therefore fewer higher-order lateral branch tips and scion type 3S had fewer branch tips capable of flowering (Figure 3).

Table 3. Adjusted open-pollinated family means (and standard errors) for female strobili per cluster and female strobili per graft and three male strobilus traits: pollen per graft, male strobili per cluster and male strobili clusters per graft.

Family ID	Female Strobili per Cluster	Female Strobili per Graft	Male Strobili per Cluster	Male Strobilus Cluster per Graft	Pollen per graft (ml)
A	1.33(±.086)	4.02(±.81)	6.45(±1.83)	0.98(±.76)	1.65(±2.53)
B	1.33(±.076)	5.94(±.85)	8.01(±1.08)	4.12(±.72)	10.03(±2.41)

Table 4. Male flowering response in March 1991, 26 months after grafting. Means and standard errors are shown for pollen quantity per graft number of male strobili per cluster and number of clusters per graft. Scion type differences were statistically significant at the 20% level in this study.

	1991 Study 26 Mo. After Grafting			1981 Study 33 Mo. After Grafting	
Scion Type	Pollen per Graft (ml)[1]	Male Strobili per Cluster	Male Strobilus Cluster per Graft	Scion [2] Age	Male Strobilus Cluster per Graft
1	3.8(±3.4)	6.07(±2.20)	1.90(±1.03)	1	.02 a
3S	3.3(±3.6)	8.67(±1.35)	1.35(±1.16)	4	.97 b
3L	9.3(±3.7)	4.76(±1.65)	4.60(±1.14)	8	.87 b
8	7.3(±3.5)	10.12(±1.58)	2.05(±1.07)	12	2.58 b

[1] 3.7 male strobili equal 1 ml fresh pollen

[2] Data from Greenwood (1984); scion age effects were statistically significant at .05 level; letters denote differences detected by Duncan's multiple comparison test.

In both the 1981 and 1991 studies, it would also appear that female strobilus induction techniques have a side effect on male strobilus production. We observed that heavy pollen production occurs when a graft's crown produces many higher-order laterals which are competent to flowering.

Comparison between 1991 and 1981 scion age studies

A comparison between the two studies suggests that the increased flowering in 1991 is due to improvements in accelerated breeding methodology. There are important similarities between the studies: 1) both studies were conducted in the same location in Hot Springs, Arkansas, 2) both sets of families were sampled from the same North Carolina Coastal Plain seed orchard and 3) both studies were tended by the senior author.

The families were not the same in each study but this seems unlikely to wholly account for the difference. The 1981 study sampled scion randomly from five half-sib families from operational full-sib genetic tests and the 1991 study sampled two additional open-pollinated families, chosen for putative "poor" and "good" flowering ability (J. Hunt, Weyerhaeuser Company, pers. comm.). Family differences may account for some variation between studies but one would expect the family effect to be larger (Table 3) if this were the sole basis for the fourfold increase in female strobilus production.

Refinements in accelerated breeding technology, which have occurred over a decade of steady practice, are thought to account for the difference. There are at least two important refinements. First, gibberellin is now applied as close to the base of the bud as possible, rather than at a constant distance from the bud. Gibberellin placement depends on the morphology of the bud rather than on a set distance from the bud tip. Secondly, the bud that forms in late summer must remain resting or quiescent until spring. This is critical to obtaining a treatment response. If watering or lighting is inadvertently changed, the resting bud may elongate, losing its treatment response for the following spring. It appears that accelerated breeding technology has continued to evolve to the point that breeding juvenile selections is no longer an obstacle.

SUMMARY AND CONCLUSIONS

Juvenile loblolly pine selections can be bred on the same schedule as older selections; there were four times as many female strobili at all ages compared to an earlier study conducted in 1981. These improvements in female strobilus induction technology have removed the adverse impact of scion age on accelerated loblolly pine breeding programs. Controlled-pollination would be more cost-effective if there were also refinements which increased the numbers of female strobilus on a branch cluster basis rather than on a whole-crown basis.

For all scion types except age one, breeding can be completed in four years rather than in five years under the abbreviated breeding schedule. We expect the shorter breeding schedule to reduce the need for 6-meter ceilings in indoor breeding orchards. Routine use of the abbreviated breeding schedule will depend on the availability of pollen at 14 months after grafting. Our next step will be to test same-year grafting and pollen induction treatments.

On a conventional five-year breeding schedule, selection age did not have an effect on pollen production. However, for age three selections, height at selection was more important than age at selection age for male strobilus production. If this proves to be consistent, then there may be an additional opportunity to reduce the breeding schedule by another year since pollen nor female strobili are limiting for three-year selections.

LITERATURE CITED

Bramlett, D. L., F. E. Bridgwater and F. R. Matthews. 1985. Theoretical impact of pollen viability and distribution on the number of strobili to use for controlled pollinations in loblolly pine. pp. 194-203. In: Proceedings 18th South. For. Tree Improv. Conf., Long Beach, MS.

Greenwood, M.S. 1981. Reproductive development in loblolly. II. the effect of age, gibberellin plus water stress and out-of-phase dormancy on long shoot growth behavior. Am. J. Bot. 68:1184-1191.

Greenwood, M. S. 1984. Phase change in loblolly pine: shoot development as a function of age. Physiol. Plant. 61:518-522.

Greenwood, M. S., C. C. Lambeth and J. L. Hunt. 1986. Accelerated breeding and potential impact upon breeding programs. pp. 39-43. In: Proceedings workshop on advanced-generation: current status and research needs. Louisiana Agricultural Experiment Station, Southern Cooperative Service Bulletin No. 309.

Li, Bailian, C. G. Williams, W. C. Carlson, C. A. Harrington and C. C. Lambeth. Gain efficiency in short-term testing: seedling height versus shoot components. Manuscript in review.

Poethig, R. S. 1990. Phase change and the regulation of shoot morphogenesis implants. Science 250:923-930.

van Buijtenen, J. P. 1986. An integrated accelerated breeding system. pp. 63-66. In: Proceedings workshop on advanced-generation: current status and research needs. Louisiana Agricultural Experiment Station, Southern Cooperative Service Bulletin No. 309.

Williams, C. G. 1987. Influence of shoot ontogeny on juvenile-mature correlations in loblolly pine. For. Sci. 33:411-422.

ACKNOWLEDGEMENTS

FOLIAR NUTRIENT VARIATION IN LOBLOLLY PINE SEED ORCHARDS

P. L. Wilcox[1], H. L. Allen[2], J. B. Jett[3]

Keywords: Pinus taeda L., seed orchard, foliar nutrient, nutrient balance, flower initiation.

INTRODUCTION

In order to justify financial investment, seed orchards must become productive as soon as possible and maintain a high level of production (Talbert et al. 1985). A variety of factors may be manipulated by managers to achieve these objectives. One such factor, nutrition, is routinely improved via use of fertilizers. The current approach to orchard nutrition usually entails soil tests to identify possible nutrient deficiencies, followed by addition of one or a combination of nutrients such as nitrogen, phosphorous, potassium, calcium, and magnesium to promote vegetative growth. Once an orchard is producing seed, nitrogen is often applied immediately prior to seed cone bud initiation to increase the incidence of initiation. The biochemical mechanisms involved in seed cone initiation are not well understood, but may be in part influenced by arginine levels in vegetative tissues (Jackson and Sweet 1972, Ross and Pharis 1985). Arginine is in turn dependent upon nitrogen availability: when uptake exceeds use, arginine levels increase thus favoring formation of reproductive tissue (Ebell and McMullan, 1970).

In contrast to seed orchards, forest managers frequently use foliar nutrient assays to reflect nutrient status of loblolly pine (Pinus taeda L.) plantations. Of importance are both the levels of the individual macronutrients (N, P, K, Ca, and Mg), and nutrient balance, as evidenced by the development of a Diagnostic and Recommendation Integrated System (DRIS) (Beaufils, 1973) for forest tree nutrition (Hockman and Allen, 1990). The technique has recently been applied to Fraser fir (Abies fraseri Pursh (Poir)) seed orchards in the southeastern United States by Arnold (1988).

[1] Scientist, Genetics and Tree Improvement, Forest Research Institute, Rotorua, New Zealand.

[2] Director, Forest Nutrition Cooperative, Department of Forestry, North Carolina State University, Raleigh, NC.

[3] Professor and Associate Director, North Carolina State University Tree Improvement Cooperative, North Carolina State University, Raleigh, NC.

The objectives of this study were:

(i)   to determine the if foliar nutrient concentrations and balances in seed orchards differed according to clone and season,

(ii) to determine if flower initiation was related to foliar nutrient status, and

(iii) to examine results in light of existing approaches in orchard nutrition, and identify areas requiring further research.

METHOD AND RESULTS

The study was composed of two data sets. The first data set was from a joint NCSU Tree Improvement/Nutrition Cooperative's project. Foliar nutrient data collected from two orchards: a Coastal Plain site in Georgia, and a Piedmont site in South Carolina. Foliar macronutrients (N, P, K, Ca, Mg) were assayed bimonthly for a twelve month period from five clones at the coastal plains site, and ten clones at the Piedmont site. At both sites, three ramets were sampled per clone. Clones were not common across sites. No counts of female flower initials were taken. Data were analyzed as a randomized complete block design, with clones and ramet planting date as random effects. Nutrient ratios as well as the individual nutrients were included as dependent variables. Significant differences among clones occurred across dates on both sites for N and Mg, and for N-P, Mg-P and Mg-N ratios. Fluctuations in nutrient levels and balances across sampling dates were also noted, with virtually all clones showing the same pattern despite some rank changes across dates. Both the seasonal pattern and range differed from patterns exhibited in loblolly pine plantations (e.g., Adams et al. 1987, McNeil et al. 1987), possibly resulting from more intensive fertilization practice in orchards. Individual nutrients and nutrient ratios were also compared to plantations. Commonly added elements such as nitrogen were generally higher than the recommended critical limits for plantations (Hockman and Allen 1990); with less frequently added elements such as calcium and magnesium (in the Piedmont site) much lower than would be accepted in a plantation. As a result, nutrient balance was also at variance with plantations – particularly those involving Ca and Mg with other elements, where ratios were also much lower than considered adequate for plantations.

The second data set came from an earlier study, some of which has been reported by Greenwood (1980). This data set consists of foliar macronutrient assays from two orchards: Aliceville, AL, and Washington, NC. Foliar nutrients were obtained from three clones at each site for one date only: winter 1976. Female cone initials were counted the following summer. Fertilizer treatment varied, with a total of 13-15 ramets/clone measured per site. Analysis of variance was used to detect differences in cone initiation between clones. Despite large differences in means for both sites, clone means were not significantly different. Correlation analysis showed significant relationships

(at the 10% level) between flower counts and Ca, Ca-P and Ca-K for between two to three out of the six clones measured. Where calcium levels and/or ratios were high, negative correlations occurred, and the reverse being the case with low calcium levels and/or ratios. In comparison with critical limits for plantations, added elements such as N were higher than deemed necessary.

IMPLICATIONS

This study has a number of potential implications for seed orchard nutrition. First the existing practice of using soil tests as a diagnostic tool may not be detecting problems associated with nutrient imbalances and/or deficiencies. Such problems may be manifested directly in reduced cone initiation, or indirectly via health problems associated with nutrient imbalance. Secondly, current fertilization practices may be creating imbalances through the frequent application of N and possibly P and K. A further implication of this study is the large difference between orchards and plantations: the more intensive approach to orchard nutrition may be increasing the potential for nutrient problems via imbalance. With the impending development of the third generation of improved loblolly pine, as well as increased demand from a limited number of (higher quality) families, managers will again be in the position of maximizing orchard productivity as early as possible, that more attention to orchard nutrition will be required in future.

RECOMMENDATIONS

Based upon the above results, and implications of this study, several recommendations can be made. Foliar nutrient assessment procedures should be adopted as standard management practice, with adequate consideration given to clonal variation and timing of foliar sampling. Simultaneously, a diagnostic system should be developed based upon foliar nutrition similar to that of plantations (e.g., Hockman and Allen 1990), as the plantation values may be inadequate for seed orchard situations. This will allow more effective management of orchard nutrition, with more attention given to non-added nutrients and nutrients balance. Secondly, further examination of the role of calcium in female strobili initiation is warranted. While the data presented in this study are far from conclusive, we feel the results are sufficient to justify a more detailed examination. Furthermore, the study highlighted a general lack of research regarding physiological mechanisms involved in strobili initiation. Such knowledge will ultimately be required if strobili production is to be maximized.

LITERATURE CITED

Adams, M. B. and H. L. Allen. 1985. Nutrient proportions in the foliage of semi-mature loblolly pine. Plant and Soil 86:27-34.

Adams, M. B., R. G. Campbell, H. L. Allen, and C. B. Davey. 1987. Root and foliar nutrient concentrations: effects of season, site and fertilization. For. Sci. 33(4):984-996.

Arnold, R. J. 1988. Foliar mineral nutrient diagnosis with DRIS for identifying nutritional influences of female cone production in fraser fir. M. S. Thesis, Dept. of Forestry, North Carolina State University, Raleigh, NC.

Beaufils, E. R. 1973. Diagnosis and recommendation integrated system (DRIS). A general scheme for experimentation and calibration based on principles developed from research in plant nutrition. Soil Sci. Bul. 1. Univ. of Natal, S. Africa. 132 pp.

Ebell, L. F. and E. E. McMullan. 1970. Nitrogenous substances associated with differential cone production responses of Douglas fir to ammonium and nitrate fertilization. Can. J. Bot. 48:2169-2177.

Hockman, J. N. and H. L. Allen. 1988. Nutritional diagnoses in loblolly pine stands using a DRIS approach. in S. P. Gessel, D. S. Lacate, G. F. Wheetman and R. F. Powers. Eds. Sustained Productivity of Forest Soils. Proceedings of the 7th North American Forest Soils Conference, University of British Columbia, Faculty of Forestry Publication, Vancouver, BC. 525p.

Jackson, D. I. and G. B. Sweet. 1972. Flower initiation in temperate woody plants: A review based largely on the literature of conifers and deciduous fruit trees. Hort. Abstr. 42:4-24.

McNeil, R. C., R. Lea, R. Ballard, and H. L. Allen. 1988. Predicting fertilizer response of loblolly pine using foliar and needle-fall nutrients sample in different seasons. For. Sci. 34(3):698-707.

Ross, S. D. and R. P. Pharis. 1985. Promotion of flowering in tree crops: different mechanisms and techniques with special reference to conifers. P. 383-397 in Attributes of Trees as Crop Plants. Cannel, M.G.R. and J. E. Jackson (eds). Institute of Terrestrial Ecology. England.

Talbert, J. T., R. J. Weir, and R. D. Arnold. 1985. Costs and benefits of a mature first generation loblolly pine tree improvement program. J. For 83(3):162-166.

GAINS FROM RUST RESISTANT ORCHARDS ESTABLISHED WITH SEEDLINGS

E.G. KUHLMAN AND H.R. POWERS[1]

Abstract. Sixteen years of experience establishing seedling seed orchards with progeny that were disease-free after inoculation with the fusiform rust organism has provided a number of benefits not usually derived from clonal orchards. Some half-sib seedling seed orchard trees from resistant mother trees produce progeny with considerably greater resistance than that of the original mother tree. Seedlings derived from controlled crosses between some resistant families have produced some orchard trees with very highly resistant progeny. Families with superior growth characteristics, but with moderate to little rust resistance, have been improved by selecting orchard trees that have both good growth and high resistance. Families with unique sources of resistance, but inferior growth characteristics, have also been improved by selecting orchard trees with improved growth as well as unique rust resistance. The average level of rust resistance is higher in orchards established with seedlings than in those established with clones.

Keywords: Cronartium quercuum f. sp. fusiforme, loblolly pine, Pinus taeda, seedling seed orchard.

INTRODUCTION

Disease resistance offers the best means of limiting damage by fusiform rust (caused by Cronartium quercuum (Berk) Miyabe ex Shirai f. sp. fusiforme) in young southern pine plantations. Both clonal and seedling seed orchards (SSO) of rust-resistant loblolly pines (Pinus taeda L.) are being developed cooperatively by the USDA Forest Service and the Georgia Forestry Commission (USFS-GFC). One of the primary reasons for adopting the SSO approach was to increase heterogeneity in the rust resistant material.   In the mid-1970's only a limited number of resistance sources had been identified.

METHODS

Powers and Kraus (1983) utilized a multi-step procedure for selecting crop trees for the SSO. First, loblolly pine seedlings were inoculated by the Concentrated Basidiospore Spray (CBS) system (Matthews and Rowan 1972). Our standard measure of familial resistance is the disease ratio (DR), which is computed by dividing the percent of seedlings with galls in the test family by that of the standard susceptible control. Test families with a DR > 0.70 are considered susceptible, those with a DR $\leq$ 0.70 are resistant (Kuhlman and Powers

1988). Susceptible families were discarded. In resistant families, seedlings with galls were culled. Healthy survivors from 14-16 resistant families were planted in orchard blocks at close spacings (1,418 trees per ha). All seedlings that became infected in the field were rogued. Additional roguing was needed to prevent crowding. Uninfected trees were rogued on the basis of poor growth or form. In an ongoing effort when an orchard tree produces seeds, its progeny are tested by the CBS system. Seed-producing trees are rogued by DR to a final density of no more than 119 trees per ha. In all, 92% of the original trees will be rogued.

This paper summarizes some of the positive results from the 16-year cooperative effort involved in the development of USFS-GFC loblolly pine SSO's.

Information presented in this paper is derived from seven SSO blocks established in 1975, 1976, and 1977. Second-generation progeny make up the SSO blocks. Those progeny were first screened in the CBS system and seedlings with galls were rogued. Rust-free survivors were used to establish the orchard blocks. Roguing in the orchard blocks has been done annually since the trees were 5 years old. Until the trees produced seeds, silvicultural characteristics were used to select residuals. Progeny of 429 trees from these seven blocks have been tested in the CBS system. Some results from those tests are presented here.

RESULTS AND DISCUSSION

Progeny from 429 trees from these seven loblolly SSO blocks have been screened in the CBS system, and 75% of these trees have produced resistant progeny according to the 0.70 DR. The statistical validity of the 0.70 DR as the criterion for separating resistant and susceptible families has been demonstrated in other studies (Kuhlman and Powers 1988, 1991).

The relative resistance of individual SSO trees, as indicated by the DR value, is helpful in deciding which trees to retain in the orchard and which ones to rogue. Three resistance categories were set up: susceptible with a DR > 0.70; resistant with DR > 0.30 to 0.70; and highly resistant with a DR $\leq$ 0.30.

Trees with 10-5 or 29R as the maternal parent usually produced resistant progeny and less frequently had highly resistant or susceptible progeny (Table 1). Trees from controlled pollinations of 29R with 10-5 have 14 progeny rated highly resistant and 14 rated resistant. Evidence is mounting that resistance in 29R is of a different type than that in 10-5, since progeny of these sources vary in response to virulent, single-gall isolates (Kuhlman, 1989, 1992). SSO trees with highly resistant progeny probably have resistance genes from both parents. Therefore, identifying different types (mechanisms) of resistance should be beneficial for determining which crosses will combine different resistance types and thus provide highly resistant progeny.

Progeny from 45 SSO trees with 11-20 as the maternal parent have been tested in the CBS system (Table 1). Six trees produced highly resistant progeny, 36 trees produced resistant progeny, and three produced susceptible progeny. Most trees infrequently had highly resistant progeny. Trees from

families 10-6 and 29R x A (Arkansas) more frequently had susceptible progeny than did the other families.

Table 1. The relative resistance of seedling seed orchard trees from 10 families indicated by the average disease ratio (DR) of their progeny in CBS tests.

Family	Highly resistant (DR < 0.30)	Resistant (DR > 0.30 < 0.70)	Susceptible (DR > 0.70)
	No. of trees in category (average disease ratio)		
10-5 x W	7 (0.19)	52 (0.48)	3 (0.77)
29R x W	1 (0.26)	16 (0.58)	3 (0.85)
29R x 10-5	14 (0.20)	14 (0.55)	-----
11-20 x W	6 (0.20)	36 (0.49)	3 (1.09)
42R x W	1 (0.24)	21 (0.48)	-----
T601 x W	------	13 (0.52)	2 (0.74)
10-6 x W	1 (0.29)	15 (0.59)	10 (0.82)
29R x 42R	4 (0.23)	9 (0.47)	-----
29R x 4625-3	1 (0.26)	8 (0.52)	2 (0.80)
29R x 1495-35	1 (0.26)	15 (0.49)	9 (0.78)

Family 7-56 is widely known for its superior growth. Previously, Miller and Powers (1983) reported a 75% rust infection level in CBS tests, but 55% infections in the field. Some SSO trees from half-sib family 7-56 have retained the good growth of the family while having higher levels of resistance (Table 2).

Table 2. Rust susceptibility and relative growth of nine trees from half-sib family 7-56 in the seeding seed orchard.

Tree	Disease Ratio (DR) of progeny	Annual Diameter growth (cm.)[a]
153-517	0.30	2.49
155-042	0.35	2.22
155-148	0.39	2.39
158-569	0.38	2.41
158-625	0.40	2.69
154-192	0.42	2.02
154-208	0.45	2.30
154-290	0.46	2.29
157-234	0.59	2.48
7-56	0.87	----

[a] dbh/age

Family 11-20 is a unique resistance source (Kuhlman 1992), but clones of 11-20 have been removed from some tree improvement programs because of poor growth characteristics. SSO trees from half-sib family 11-20 have yielded resistant progeny with a range of disease ratios (Table 3). These SSO trees have good growth rates that also should be passed on to their progeny. The SSO approach presents the opportunity to preserve the unique 11-20 resistance type by selecting trees with that resistance and with good growth.

Table 3. Rust susceptibility and relative growth of 12 trees from half-sib family 11-20 in the seedling seed orchard.

Tree	Disease ratio (DR) of progeny	Annual diameter growth (cm)[a]
156-316	0.05	2.29
153-362	0.14	2.16
156-137	0.28	2.10
153-510	0.28	1.75
151-464	0.29	2.55
156-203	0.32	2.29
151-026	0.32	1.94
152-122	0.34	2.30
151-654	0.37	1.26
152-196	0.41	2.38
152-329	0.43	2.49
151-620	0.47	2.24
11-20	0.40	

[a] dbh/age

Progeny from SSOs are more rust resistant in CBS tests than those from clonal orchards (Powers and Kraus 1986). Since we have expanded our clonal orchard with ramets from the SSO, these differences will probably soon disappear.

CONCLUSIONS

The SSO provide several advantages over the more traditional clonal orchard. Foremost is the chance to diversify the genetic base in the orchard with a heterogeneous seedling poulation. Second, we can identify highly resistant trees. Third, we can select trees with increased resistance that maintain the superior growth characteristics of their maternal parent. Fourth, unique resistance genes can be incorporated in trees with good silvicultural characteristics to save resistance genes that might otherwise be discarded. The major drawback to the SSO is that this method is labor intensive.

LITERATURE CITED

Kuhlman, E.G. 1989. Virulent isolates of Cronartium quercuum f. sp. fusiforme may identify different resistance genes. 7 pp. In Proceedings of the 20th Southern Forest Tree Improvement Conference, Charleston, South Carolina, June 26-30, 1989.

Kuhlman, E.G. 1992. Interaction of virulent single-gall isolates of Cronartium quercuum f. sp. fusiforme and resistant families of loblolly pine. (In preparation)

Kuhlman, E.G. and H.R. Powers, Jr. 1988. Resistance responses in half-sib loblolly pine progenies after inoculation with Cronartium quercuum f. sp. fusiforme. Phytopathology 78:484-487.

Kuhlman, E.G., and H.R. Powers, Jr. 1991. Symptom expression among half-sib slash pine progenies after inoculation with Cronartium quercuum f. sp. fusiforme. Can. J. For. Res. _____.

Matthews, F.R., and S.J. Rowan. 1972. An improved method for large-scale inoculations of pine and oak with Cronartium fusiforme. Plant Dis. Rep. 56:931-934.

Powers, H.R., Jr., and J.F. Kraus. 1983. Developing fusiform rust-resistant loblolly and slash pines. Plant Dis. 67:187-189.

Powers, H.R., Jr., and J.F. Kraus. 1986. A comparison of fusiform rust-resistant loblolly pine seed sources. S. J. Appl. For. 10-230-232.

GENERAL SESSION III

BREEDING AND PROPAGATION

RECURRENT SELECTION, MATING DESIGN, AND
EFFECTIVE POPULATION SIZE

H. Kang[1]

Abstract. -- Many contemporary breeders are building founder breeding populations, and there is a need to learn about the potential long-term consequences of their breeding activities. In this paper impacts of recurrent selection, mating design, and effective population size on dynamics of breeding populations are discussed. A theoretical model of a single-loci population is used to highlight the relevant information on long-term tree breeding.

Keywords: breeding population, mating design, allele fixation, allele frequency

INTRODUCTION

Long before Darwin (1809-1882) and Mendel (1822-1884) provided empirical evidence of the process of evolution and mechanism for inheritance, humans had domesticated animals and crops by practicing artificial selection. These domestication processes demonstrated the human ability to select for modified forms of plants and animals. Likewise, artificial selection remains an important feature of forest tree breeding as contemporary tree breeders domesticate and breed trees. To many tree breeders, however, selection represents only a subset of activities associated with the broader objective of managing genetic resources. Tree breeders are interested not only in maximizing immediate genetic gain, but also in learning the potential long-term genetic consequences of various breeding activities. This reflects increasing awareness that whatever tree breeders do today will influence future tree breeding for a long time: what is good for today may not necessarily be good for tomorrow. Decisions made or not made now will limit future options.

Three main questions contemporary tree breeders may ask about long-term breeding are: (1) How large should the overall breeding population size be? (2) How should the breeding population be structured? and (3)What are the long-term consequences of using current breeding techniques? To address these questions, we need to consider dynamics of breeding populations over many generations. Although most forest tree species cannot be used for this purpose, it is possible to learn about population dynamics by means of theory and empirical study of fast generation turn-over plants, including some tree species. These alternative means do not allow us to predict specific conditions of the breeding population at some time in the future, but they do

---

The main objective of this paper is to address some aspects of the above three long-term tree breeding questions. I will: (1) review some basic concepts of artificial selection and effective population size; and (2) discuss recurrent selection, mating design, and population size using a single-loci model.

SELECTION, MATING DESIGN, AND EFFECTIVE POPULATION SIZE

Single cycle of selection:

Quantitative geneticists often consider the change in the population mean ($\Delta\mu$) as the response to a single cycle of selection. This quantity is often equated to the genetic gain -- i.e., $\Delta\mu = \Delta G = ih^2\sigma_p$, where i, h^2, and σ_p represent intensity of selection, heritability, and phenotype standard deviation of the parental population, respectively. In theoretical population genetic models, on the other hand, the change in the frequency of a selectively favored allele (Δq) is often used, where q represents the allele frequency before selection. In a single-loci model, Δq is a function of both allele frequency, selection coefficient(s), and measure of dominance (h). Single loci model means that the character of interest is controlled by many independent loci -- i.e., no linkage and no epistasis. The parameter used in quantitative genetics and the parameter used in population genetics, $\Delta\mu$ and Δq, are related through the expression (Falconer 1981),

[1] $\qquad s \approx i\alpha$,

where s, i, and α represent selection coefficient, selection intensity, and the standardized distance between two homozygotes, respectively. This relationship assumes that the environmental effect is normally distributed. For example, Fisher's (1918) infinitesimal model deals with the case where the number of loci influencing the character subjected to selection is effectively infinite, and the environmental effect is assumed to be normally distributed. Even if this normality assumption does not hold, i in [1] could be replaced by the ratio between the ordinate at the truncation point to the proportion selected, as long as truncation selection is made and the gene effects on the character are additive between loci (Kimura and Crow 1978).

We may question the value of [1] because the assumptions used to define the equation are unrealistic. There are many unknowns regarding genetic properties of the population, and [1] cannot be used in a predictive fashion. For example, knowing $\Delta\mu$ will say nothing about Δq. In most quantitative traits, we do not know the number of loci influencing the character and their gene actions. However, [1] is useful because it implies that there is a one-to-one correspondence between s and α for given i, or between s and i for given α. Therefore, when we compare other factors such as mating design with respect to one parameter, say $\Delta\mu$, then we can expect the same trend to hold with respect to Δq.

Multiple cycles of selection and Selection limit

When repeated artificial selection is applied to a population, the population may cease to respond to the selection at some point. The population could reach this selection limit for many different reasons such as (Eisen 1980): (1) fixation of all loci affecting the trait; (2) overdominance for the trait; (3) artificial selection opposed by natural selection; (4) undesirable recessive genes at low frequency; (5) negative genetic correlation between component characters; (6) genotype by environment interaction; and (7) tight linkages. For most of these cases, populations will have genetic variances at the limit and will respond to reverse selection. When all the loci affecting the trait are fixed with one allele, then there will be no genetic variance, and reverse selection will not yield a response.

Many experimental results on recurrent selection are available. Wright (1977) made an extensive review of this subject. Some notable studies of selection limit are: Jones et. al. (1968) for fruit fly, Roberts (1966a,b, 1974) and Eisen (1972, 1974) for mouse, and Enfield (1974, 1977) for flour beetle. These experiments were designed to test the influence of variance effective population size (N_{ev}) and selection intensity (i) (or population structure). Some important conclusions from these studies are: (1) The total response to selection increases when the population size increases. (2) For a fixed population size, the total response increases when the selection intensity increases. (3) The estimates of the time taken to reach the selection limit in general do not agree with the theoretical prediction by Robertson (1960). There are other landmark experiments. Dudley (1977) has shown that the percentage of both oil and protein in maize showed no sign of approaching limits after 76 generations of upward selection. Wright (1977) called the findings of Payne (1918) striking, where a population drawn from a single wild *Drosophila* female responded to selection (scutellar bristle) for more than 35 generations.

Robertson (1960) showed that the "half-life" (number of generations necessary to reach the allele frequency half way to the selection limit) would vary between $1.4N_{ev}$ and $2N_{ev}$. Empirical results showed that this prediction did not work, but we may use these expressions to discuss forest tree breeding. Consider a species with a generation turn over period of 10 years. If 50 individuals are selected every generation, it would take at least 70 generations or 700 years to reach the half-life. It would take a long time before a selection limit is reached. Should tree breeders be concerned about selection limit? It is unrealistic to begin a breeding program with the idea that some day the breeding population will reach its selection limit. Breeders may consider selection limit as a conceptual restriction. It is useful to know that a chosen strategy does not lower the selection limit. Given this restriction breeders may choose the strategy which would allow the maximum short-term genetic gain. The parameters $u(q)$ and $t(q)$ themselves could be used in developing breeding strategies. Understanding these parameters would help in determining necessary breeding population sizes and in designing the structure of breeding populations. It would also offer alternate views on short-term breeding techniques. It is well known that the selection limit is usually smaller than that projected by the gain at the beginning of the breeding program (Bohren 1975). Learning the causes of such discrepancies would greatly help breeders to appreciate the factors that influence population dynamics.

The basic theory of limits to artificial selection was first set forth by Robertson (1960). To develop the theory Robertson used Kimura's (1957) expression for the ultimate probability of allele fixation u(q). This probability is a function of the initial allele frequency (q), variance effective population size (N_{ev}), and selection coefficient (s) such that,

$$[2] \quad u(q) = \frac{\int_0^q G(x)dx}{\int_0^1 G(x)dx},$$

where $G(x) = \exp[-2N_{ev}s(2h-1)x(1-x) - 2N_{ev}sx]$.

    q represents the initial allele frequency,
    N_{ev} represents variance effective population size,
    h represents the degree of dominance, and
    s represents the selection coefficient.

Under an infinite population size model, the selection will not fix the favorable allele when overdominance gene action controls the locus, and u(q)=0. In [2], however, there is always the possibility of chance fixation of alleles ($u(q) \neq 0$), and u(q) is a useful measure regardless of the gene action. The single-loci model also assumes independence among loci (no linkage and no epistasis), discrete generation, and constant N_{ev} and s. Given all these assumptions, the dynamics of the population is completely explained by the three parameters, q, N_{ev}, and s. In artificial breeding where truncation selection is used, we may replace s with *i* and α (Equation [1]).

Some additional conclusions of Robertson (1960) are: (1) For small populations, the advance due to selection is greatest when 50% of the population is selected. This was also predicted by Dempster (1955), and Cockerham and Burrows (1980). (2) For small additive allele effect (α in Equation [1]), if $N_{ev}i$ is small, then the total response is approximately $2N_{ev}$ times the response in the first generation. Subsequently, many theories dealing with selection limit have been developed: Conflict between natural and artificial selection (James 1962, Sved 1977, Nicholas and Robertson 1980); Exact probability (Hill 1969a, Carr and Nassar 1970a,b); Finite number of gametes (Schuster and Sigmund 1989); Linkage (Hill and Robertson 1966, Gill 1965a,b, Latter 1965, 1966, Robertson 1970); Mating design (Kang and Namkoong 1979, 1980, Kang 1983); Mutation (Hill and Keightley 1988, Hill and Rasbash 1986, Keightley and Hill 1988); Overlapping generations (Emigh and Pollak 1979); Rate of response (Hill 1969b, Kimura and Ohta 1969); Self-fertilizing population and inbreeding (Bailey 1977, Hill and Robertson 1968, Robertson 1961); Structured population (Baker and Curnow 1969, Madalena and Hill 1972, Hill 1970); and Within-family selection (Dempfle 1975, Young and Skavaril 1976).

The connection between u(q) defined in the theoretical models and observed total advance from selection experiments may be interpreted as follows. The level of the limit, (i.e., the height of the plateau) will be influenced by the number of favorable alleles fixed. Therefore, the greater u(q) for the loci involved, the greater the height of the plateau is likely to be. At fixation, the population will lack genetic variance with respect to the character selected. However, empirical results have shown that when reverse selection was made at selection limit,

the population almost always responded, implying that the population had genetic variability at the limit. It was also mentioned before that empirical results did not agree with Robertson's conclusion regarding the half-life. Therefore, selection theories appear to be of limited value as means of predicting total advance from selection. These theories, on the other hand, have been extremely useful in sorting out factors that influence the selection limit. Experimental results, indeed, indicate that N_{ev} and s are two critical factors that influence the limit. Because the factors that influence the population dynamics are completely represented by three parameters, q, N_{ev}, and s, it must be possible to explain any variation in the selection regime by using these parameters. For example, the influence of the mating designs on the selection limit could be explained based on how they influenced the variance effective population size (Kang and Namkoong 1979, 1980, Kang 1983). This will be discussed in a later section.

Mating design, variance effective population size, and allele fixation

Mating design represents "rules" for arranging different control crossings. There are three different standard types of mating designs: nested, factorial, and diallel. Most of these designs allow the estimation of both additive and dominance genetic variance. There are large differences among designs with respect to the number of control matings necessary. To take the most extreme example, full-diallel requires N^2 crossings while pair mating requires N/2 crossings to complete the design, where N represents the census number of parents. Therefore, the full diallel mating requires 2N times more crossings than pair mating, and the ratio will increase as the number of selected parents increases. Using complex designs is often biologically impossible as well as costly and time consuming. Traditional mating designs assume that mating is made randomly after truncation selection. Instead of random mating, assortative mating may be used. These assortative matings could be balanced or unbalanced, where balanced mating means that all the parents have equal probability of passing the same number of alleles to the progeny gene pool. Alternatively, it is possible to assign weights to the ordered breeding values (Kimura and Crow 1978, Crow and Kimura 1979, Lindgren and Matheson 1986, Lindgren et al. 1989, Kang and Namkoong 1988, Kang 1989). These mating systems are necessarily unbalanced.

Mating designs were originally developed as means of estimating genetic parameters such as additive- and/or dominance variance. Mating design has also been used in developing selection strategies in short-term breeding. The designs offer different hierarchical structures, such as half-, full-sib family, and individuals within family, in the progeny population. This hierarchical structure provides the basis for constructing selection indices. In evaluating mating designs with respect to estimation, the sampling variance of the genetic variances are used (Nasoetion et al. 1967, Namkoong and Roberds 1974, Pepper 1983). In general, for a given number of parents, the mating design that includes the larger number of crossings will produce the smaller sampling variance. For example, Klein et al. (1973) indicated that it would take at least 400 families to estimate heritability with a standard error less than 0.1.

The predicted genetic gain under different selections has received great attention. For example, it is well known that family selection is more desirable than within-family selection when the heritability (or intraclass correlation) is low (Falconer 1981). This kind of idea has

been generalized into the form of index of family and within-family values. Because family structures can be created by the mating systems, it is possible to evaluate selection-mating design combinations (Cockerham and Matzinger 1966, Namkoong et al. 1966, van Buijtenen 1972, Lindgren 1977, Pepper and Namkoong 1978, Cotterill and Jackson 1989). Mating designs seem to have relatively little impact on the index-mating design combinations (Cotterill and Jackson 1989). For both mass selection and family selection, mating designs have relatively little impact on Δq as long the mating designs are balanced (Kang and Namkoong 1979, Kang 1983).

There are some theoretical as well as empirical findings that indirectly indicate that balanced mating designs may not differ with respect to fixation probabilities: (1) Hill and Robertson (1968) examined the effects of inbreeding in monoecious and dioecious populations when heterozygote advantage existed. They found little difference between the two populations with respect to equilibrium allele frequencies. (2) Lande (1977) found that mating system had no influence on the amount of genetic variance maintained in the model population that allowed mutation, linkage, and natural selection on a polygenic character with additive genes. (3) My unpublished results from the single-locus model showed that selfing did not change $u(q)$. (4) MacNeil et al. (1984) studied effects of mating systems in Japanese quail. In this experiment, an inbreeding and a random mating population were compared. They found that once the inbreeding population overcame the initial depression its performance level increased rapidly. (5) Cockerham and Burrows (1980) indicated that to maximize the selection limit in dioecious populations the optimal procedure would be to equal numbers recorded and selected of each sex.

The theoretical explanation for why $u(q)$ do not differ between balanced mating designs, but differ among unbalanced mating designs can be found by observing the impacts of different mating designs on variance effective population size (N_{ev}). The variance effective population size is defined as (Crow and Denniston 1988),

[3] $$N_{ev} = \frac{2N_t}{1-\epsilon+\dfrac{(1+\epsilon)s_k^2}{\mu_k}} \text{, for a monoecious population,}$$

where N_t represents the number of progeny individuals after selection,

ϵ represents deviation of parent genotypes from Hardy-Weinberg equilibrium,

μ_k represents the mean of the number of gametes transmitted by a parent, and

s_k^2 represents the sample variance of the number of gametes transmitted by a parent.

Under balanced mating, $N_{ev} = N_{t-1}$ regardless of the number of crossings involved, because $s_k^2 = \mu_k$. This holds for assortative mating also as long as they are balanced. With unbalanced mating, however, $N_{ev} < N_{t-1}$ and $s_k^2 > \mu_k$. In a recurrent selection, where the census population size (N) is kept constant, $\mu_k = 2$, for all mating designs, and the difference between balanced and unbalanced mating design originates from different s_k^2. Because N_{ev} of unbalanced mating design is always smaller than that of balanced mating design, it generates lower

probability of allele fixation (u(q)) for given q, s (or i and α), and N_{t-1}. For example, assume that the number of parents selected (N_{t-1}) is 8, then N_{ev} of full-diallel, pair mating, and selfing is 8, but that of factorial mating with 1 tester is 4.06. When the census number (N_t or N) is 16, N_{ev} of a factorial mating design with 1 tester is 4.01. In general, with 1 tester factorial,

$$N_{ev} = \frac{2N}{1-\varepsilon+\dfrac{(1+\varepsilon)N(N-2)^2}{2(N-1)^2}}, \text{ which implies that as N approaches infinity, } N_{ev} \text{ approaches 4,}$$

assuming that $\varepsilon = 0$. When N=8, q = 0.25, i = 1.755 (10% selection), α = 0.2, and additive gene action, u(q) for all the balanced mating is 0.76. For factorial mating with 1 tester u(q) is 0.54, even if the census number (N) is greater than 8. The value of u(q) is found by evaluating Equation [2] after replacing proper values listed above.

Equation [2] is not an exact solution, but has been proven to be a good approximation (Hill 1969a, Carr and Nassar 1970a,b). Balanced mating designs of u(q) calculated by using a numerical analysis (Kang and Namkoong 1979, 1980) also resulted in the same values as those obtained from Equation [2]. However, the numerical analysis of factorial mating design resulted in lower u(q) than the solutions from [2] (Figure 1). It is possible that there other than N_{ev} might influence u(q). For example, in the calculation of N_{ev}, ε (deviation from Hardy-Weinberg proportion) is assumed to be zero, which is rarely true. In any case, the discrepancy
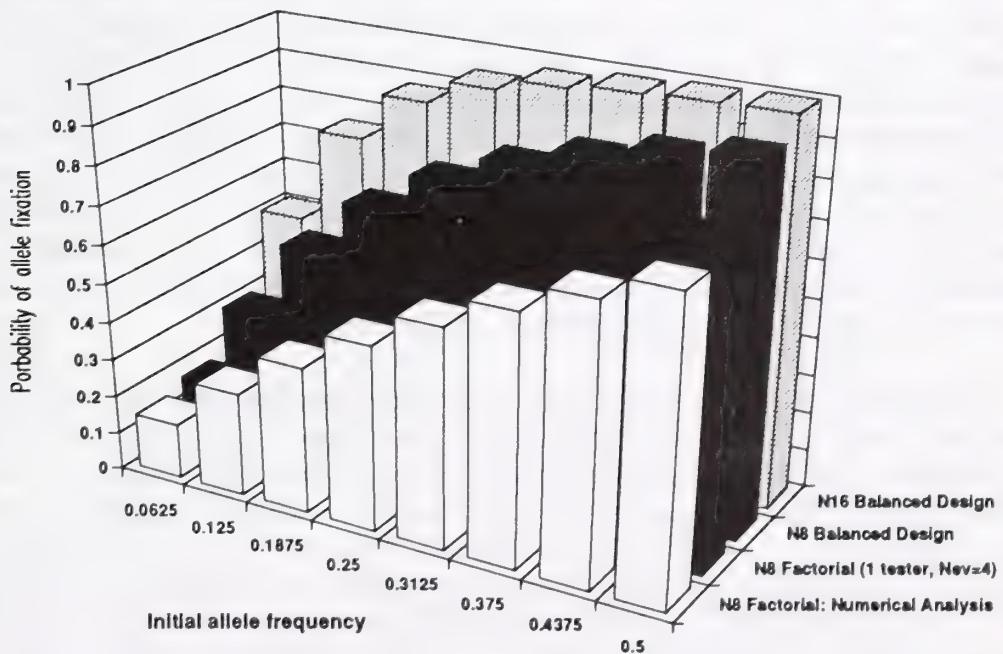
Figure 1. Ultimate probability of allele fixation (u(q)) under different initial allele frequency and population size. N# represents the census number. Initial conditions used are: α=0.2, i=1.755 (10% selection), additive gene action. The u(q) for balanced designs (N16, N8) and N8 Factorial (1 tester, Nev=4) were obtained by using Equation [2].

appears to be fairly consistent over different initial allele frequencies. Figure 1 also shows that for a moderate initial allele frequency, say q > 0.3, and N_{ev} = 16 the probability of allele fixation, u(q), is close to 1 with balanced mating. Therefore, if the allele fixation under selection is the only concern in long-term tree breeding, then the breeding population size does not need to be very large, unless the breeder wishes to save favorable alleles of very small initial frequency.

COMPARISON OF MATING DESIGNS UNDER A SINGLE-LOCI MODEL: A COMPUTER SIMULATION

In this section, I will discuss findings from a simple computer simulation experiment that compared response to selection to different mating designs. The analytic expression for u(q) in [2], or numerical analysis used in Kang and Namkoong (1979, 1980) provides information on populations at the selection limit, but does not show intermediate progress of the population. Computer simulation was made to observe the pattern of approach to selection limit when different mating designs were used.

In this experiment, the trait of interest of a population was assumed to be composed of a single locus (or many independent loci). As before, the initial conditions used were q = 0.25, i = 1.755 (10% selection), α = 0.2, and additive gene action. The genotype frequency of the initial population was set to be in Hardy-Weinberg equilibrium. From this founder population N parents were randomly sampled, and crossed according to a mating design. From the progeny population produced, N individuals were selected based on their phenotypic score. These selected individuals were used as parents for the next generation. Before control crossing, these individuals were sorted according to their phenotypic values. Therefore, pair mating in this experiment represents an assortative mating. Selfing, of course, is the most extreme form of assortative mating, regardless of the order of selected individuals. The recurrent selection was continued for 50 generations. This computer trial of 50 generations of selection was repeated 1,000 times. At each generation, the number of subpopulations with allele fixation and allele loss was determined, and average allele frequency was calculated.

As expected, all balanced mating designs approached the same selection limit near 0.75. (Figure 2a). The factorial mating design with 1 tester (N_{ev} = 4) approached a lower selection limit near 0.65. This level is actually higher than u(q) obtained from Equation [2] (0.54) and numerical analysis (0.49). Although all the balanced matings approached the same selection limit, the progress from selection was fastest with selfing, which was distantly followed by pair mating. For all practical purposes, pair mating, half-diallel, and partial-diallel may be considered the same in this experiment. The initial rates of progress from selection by both half-diallel and partial diallel were similar and slow. When compared to balanced mating designs other than selfing, the factorial mating had relatively rapid initial progress from selection, but produced lower plateau.

Numbers of populations with allele fixation or loss at different generations also show consistent results (Figure 2b). To simplify the picture, half-diallel and partial-diallel were not
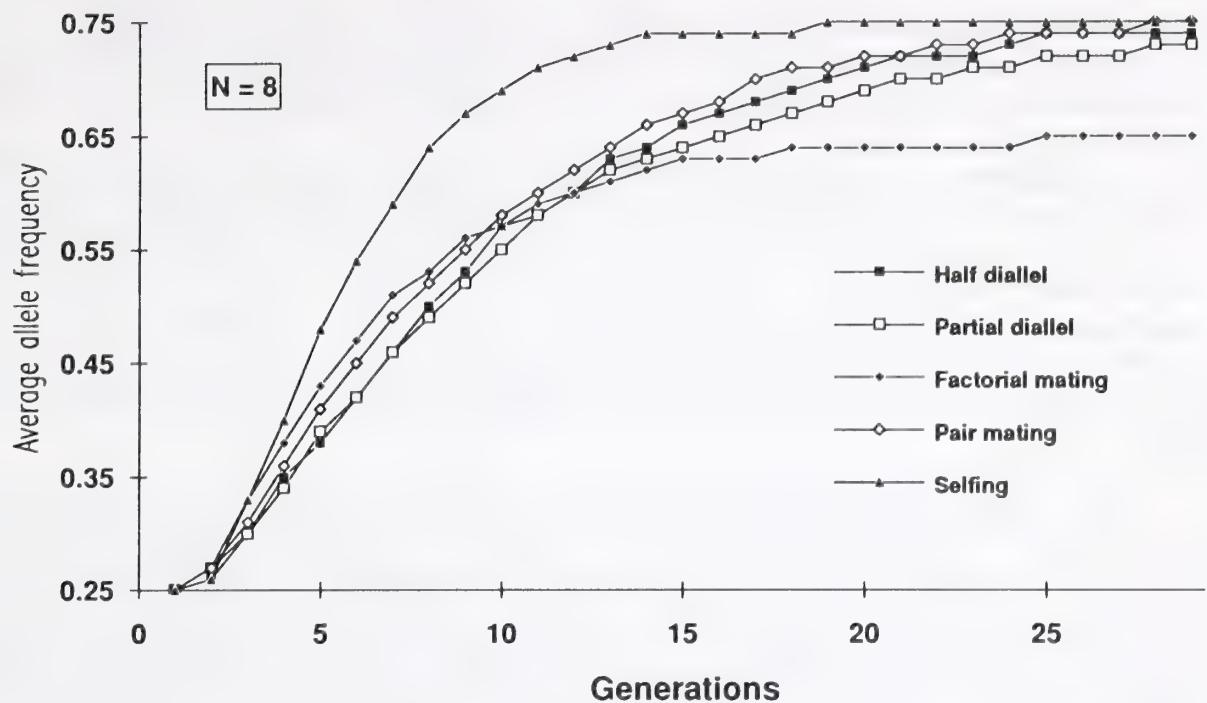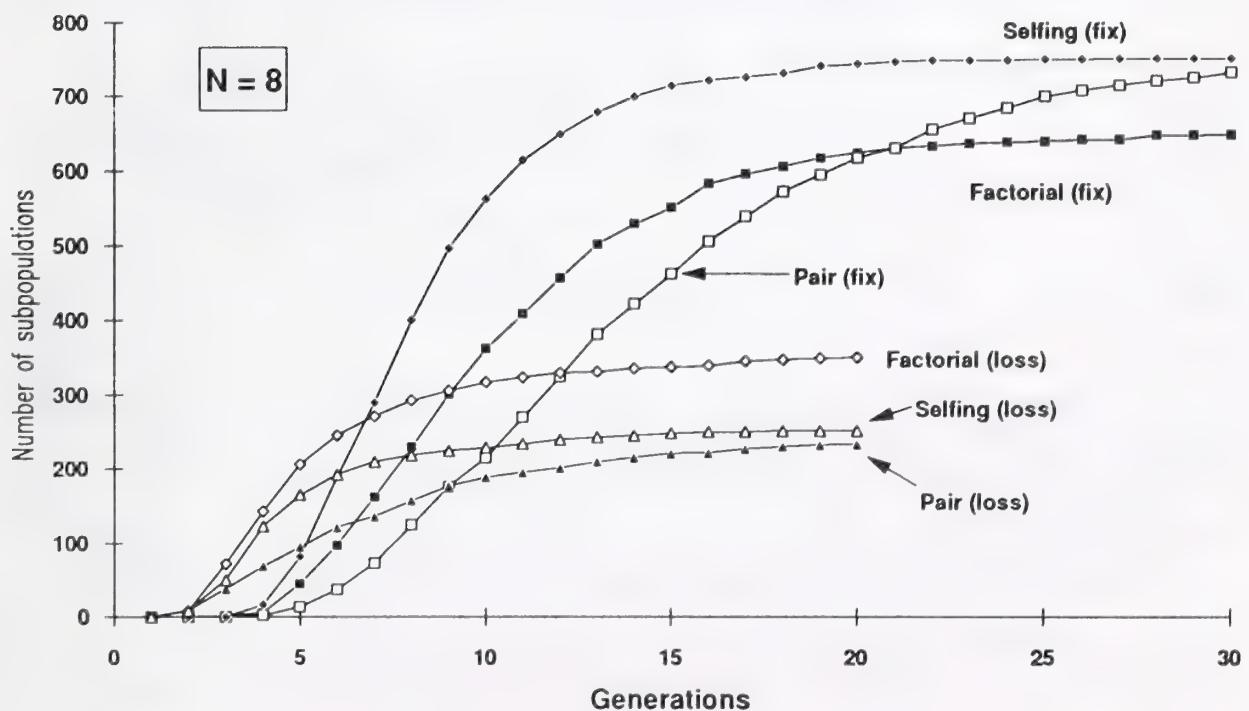
Figure 2. Results from single-loci selection simulation. **a**. Changes in the average allele frequency of 1,000 trials. **b**. Number of populations with allele fixation or loss.

included in Figure 2b. Selfing tends to have a larger number of subpopulations with allele fixation at earlier generations than pair mating. Eventually the number of subpopulations with allele fixation merged. Factorial mating also had a fairly large number of subpopulations with fixation during earlier generations, but a lower overall number of subpopulations with fixation.

Selfing also had a larger number of subpopulations with allele loss in early generations, when compared to pair mating (Figure 2b). Both have plateaus at the same level. Factorial had larger number of subpopulations with allele loss in early generations than balanced mating designs, and a higher plateau. This is primarily because of the reduced variance effective population size ($N_{ev} = 4$). The loss level, however, was lower than that expected based on loss probability $(1 - u(q))$. The number of subpopulations with allele loss is greater than that with allele fixation, which is a result of using a low initial allele frequency in this simulation ($q = 0.25$).

The average allele frequency of populations increases faster and reaches a higher plateau as the variance effective population increases (Figure 3). The number of subpopulations with allele fixation increases faster with smaller effective population size. This brings out an intriguing question in tree breeding. Which of the two parameters, average allele frequency and the number of subpopulations with allele fixation, is more important in developing a long-term breeding strategy? If we chose average allele frequency, then we would opt for larger subpopulations. On the other hand, if we maintained a large set of smaller subpopulations, we would be able to obtain populations with fast allele fixation possibility. For a given individual
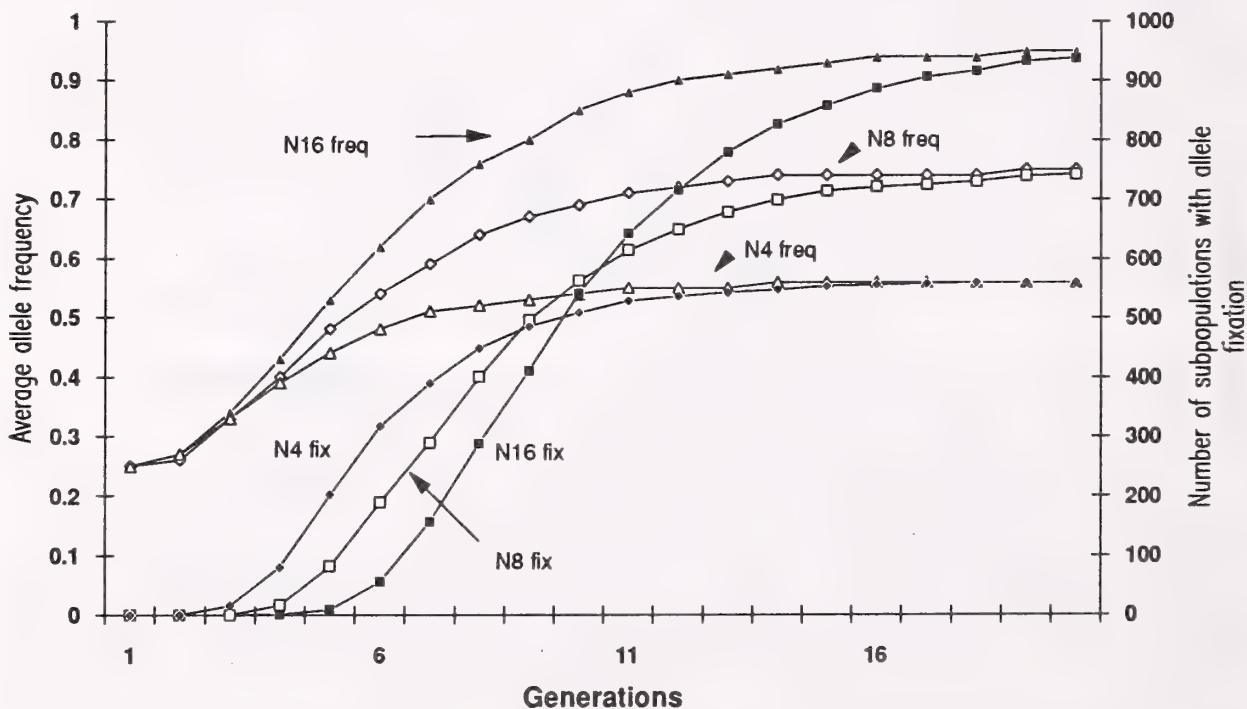
Figure 3. Average allele frequency and number of subpopulations with allele fixation under selfing.

subpopulation, the probability of allele fixation will be lower; but when all the subpopulations are combined, the overall probability of allele fixation can be maintained by hybridizing subpopulations when they reach their selection limits (Baker and Curnow 1969, Madalena and Hill 1972). As discussed before, selection limit may be viewed as a conceptual restriction, and breeders may choose the strategy that will allow the quickest response to selection. It is clear from Figure 3, that if we have a population with $N_{ev} = 1$, then the early rate of increase in the number of subpopulations with fixation will be greatest. However, $N_{ev} = 1$ implies selfing in monoecious species, which is often avoided by tree breeders.

Inbreeding would, no doubt, expose deleterious alleles in the breeding populations, and would be a source of concern for tree breeders. It is, however, desirable to purge deleterious alleles during early generations of tree breeding (Kang 1982, Kang and Nienstaedt 1987). Fisher (1965) emphatically argued for the importance of inbreeding and purging of deleterious alleles from breeding populations: "Practical breeders of farm animals are naturally deterred from a form of mating which is liable to produce animals undersized, unproductive, and prone to disease. Their reluctance is doubtless enhanced by a subconscious abhorrence of incest in their own species. Nevertheless, when an inbred line is formed from elite stock, it can contain no genes, however inferior it may appear, which were not present in its admired progenitors, nor can it hand any others on to its descendants. At the expense of some loss of appearance, and immediate utility, and with the real inconvenience of lower fertility, which may make the maintenance of such stocks difficult, the germ plasm may have been purified of many unnecessary defects, and the great boon of reliability of breeding performance gained."

This simulation shows that values of different breeding techniques vary depending on the perspectives used by tree breeders. There is a clear need to evaluate different breeding techniques with respect to long-term breeding, and then search for ways to combine short- and long-term breeding activities.

LITERATURE CITED

Bailey, T.B. 1977. Selection limits in self-fertilizing populations following the cross of homozygous lines. In: E. Pollak, O. Kempthorne, and T.B. Bailey,(eds.), Proceedings of the International Conference on Quantitative Genetics, pp. 399- 412. Ames, Iowa.

Baker, L.H. and R.N. Curnow. 1969. Choice of population size and use of variation between replicate populations in plant breeding selection programs. Crop Sci 9:555-560.

Bohren, B.B. 1975. Designing artificial selection experiments for specific objectives. Genetics 80:205-220.

Carr, R.N. and R.F. Nassar. 1970a. Effects of selection and drift on the dynamics of finite populations. I. Ultimate probability of fixation of a favorable allele. Biometrics 26:41-49.

Carr, R.N. and R.F. Nassar. 1970b. Effects of selection and drift on the dynamics of finite populations. II. Expected time to fixation or loss of an allele. Biometrics 26:221-227.

Cockerham, C.C. and P.M.Burrows. 1980. Selection limits and strategies. Proc. Natl. Acad. Sci. USA 77:546-549.

Cockerham, C.C. and D.F. Matzinger. 1966. Simultaneous selfing and partial diallel test crossing. Aust. J Biol. Sci. 19:795-805.

Cotterill, P.P. and N. Jackson. 1989. Gains expected from clonal orchards under alternative breeding strategies. Forest Science 35:183-196.

Crow, J.F., and C. Denniston. 1988. Inbreeding and variance effective population numbers. Evolution 42:482-495.

Crow, J.F., and M. Kimura. 1979. Efficiency of truncation selection. Proc. Nat. Acad. Sci. USA 76:396-399.

Dempfle, L. 1975. A note on increasing the limit of selection through selection within families. Genet. Res. 24:127-135.

Dempster, E.R. 1955. Genetic models in relation to animal breeding. Biometrics 11:535-536.

Dudley, J.W. 1977. 76 generations of selection for oil and protein percentage in maize. In: E. Pollak, O. Kempthorne, and T.B.Bailey Jr. (eds.), Proceedings of the International Conference on Quantitative Genetics, pp. 459-473. Ames, Iowa.

Eisen, E.J. 1972. Long-term selection response for 12-day litter weight in mice. Genetics 72:129-142.

Eisen, E.J. 1974. The laboratory mouse as a mammalian model for the genetics of growth. First World Congress on Genetics Applied to Livestock Production. Vol. 1, pp. 467-492, Madrid.

Eisen, E.J. 1980. Conclusions from long-term selection experiments with mice. Z. Tierzüchtg. Züchtgsbiol. 97:305-319.

Emigh, T.H. and Pollak, E. (1979) Fixation probabilities and effective population numbers in diploid populations with overlapping generations. Theor. Pop. Biol. 15:86-107.

Enfield, F.D. 1974. Recurrent selection and response plateau. First World Congress on Genetics Applied to Livestock Production. Vol. 1, pp. 365-371. Madrid.

Enfield, F.D. 1977. Selection experiments in tribolium designed to look at gene action issues. In: E. Pollak, O. Kempthorne, and T.B.Bailey Jr. (eds.), Proceedings of the International Conference on Quantitative Genetics, pp. 177-190. Ames, Iowa.

Falconer, D.S. 1981. Introduction to quantitative genetics.- 2nd ed. Longman, London and New York. 340pp.

Fisher, R.A. 1918. The correlation between relatives on the supposition of Mendelian inheritance. Roy. Soc. (Edinburgh), Trans. 52:321-341.

Fisher, R.A. 1965. The theory of inbreeding. Academic Press, New York. 150p.

Gill, J.L. 1965a. Effects of finite size on selection advance in simulated genetic populations. Aust. J. Biol. Sci. 18:599-617.

Gill, J.L. 1965b. A monte carlo evaluation of predicted selection response. Aust. J. Biol. Sci. 18:999-1007.

Hill, W.G. 1969a. On the theory of artificial selection in finite populations. Genet. Res. 12:307-343.

Hill, W. G. 1969b. The rate of selection advance for non-additive loci. Genet. Res. 13:165-173.

Hill, W.G. 1970. Theory of limits to selection with line crossing. In K. Kojima (ed.) Mathematical topics in population genetics. pp. 210-245. Springer-Verlag, N.Y.

Hill, W.G. and P.D.Keightley. 1988. Interrelations of mutation, population size, artificial and natural selection. In B.S. Weir, E.J. Eisen, M.M. Goodman, and G. Namkoong (eds.) Proc. of the 2nd Int. Conf. on Quan. Genet. Raleigh, N.C. pp. 57-70. Sinauer Associates.

Hill, W.G. and J. Rasbash. 1986. Models of long-term artificial selection in finite populations. Genet. Res. 48:41-50.

Hill, W.G. and A. Robertson. 1966. The effect of linkage on limits to artificial selection. Genet. Res. 8:269-294.

Hill, W.G. and A. Robertson. 1968. The effect of inbreeding at loci with heterozygote advantage. Genetics 60:615-628.

James, J.W. 1962. Conflict between directional and centripetal selection. Heredity 17:487-499.

Jones, L.P., R.Frankham, and J.S.F.Barker. 1968. The effects of population size and selection intensity in selection for a quantitative character in Drosophila. Genet. Res. 12:249-266.

Kang, H. 1982. Components of a tree breeding plan. In: Proceedings of IUFRO Joint Meeting of Working Parties on Genetics about Breeding Strategies including Multiclonal varieties. Sensenstein, September 6-10, 1982. pp. 119-135.

Kang, H. 1983. Limits of artificial selection under balanced mating systems with family selection. Silvae Genet. 32:188-195.

Kang, H. 1989. Inbreeding effective population size under some artificial selection schemes. II. Normal distribution of breeding values. Forest Science 35:303-318.

Kang, H. and G. Namkoong. 1979. Limits of artificial selection under balanced mating systems. Silvae Genetica 28:53-60.

Kang, H. and G. Namkoong. 1980. Limits of artificial selection under unbalanced mating systems. Theor. Appl. Genet. 58:181-191.

Kang, H. and G. Namkoong. 1988. Inbreeding effective population size under some artificial selection schemes. I. Linear distribution of breeding values. Theor. Appl. Genet. 58:181-191.

Kang, H. and H. Nienstaedt. 1987. Managing long-term tree breeding stock. Silvae Genet. 36:30-39.

Keightley, P.D. and W.G. Hill. 1988. Quantitative genetic variability maintained by mutation-stabilizing selection balance in finite populations. Genet. Res. Camb. 52:33-43.

Kimura, M. 1957. Some problems of stochastic processes in genetics. Ann. Math. Stat. 28:882-901.

Kimura, M. and J.F. Crow. 1978. Effect of overall phenotypic selection on genetic change at individual loci. Proc. Natl. Acad. Sci. USA. 75:6168-6171.

Kimura, M. and T. Ohta. 1969. The average number of generations until fixation of a mutant gene in a finite population. Genetics 61:763-771.

Klein, T.W., J.C. DeFries, and C.T. Finkbeiner. 1973. Heritability and genetic correlation: Standard errors of estimates and sample size. Behavior Genetics 3:355-364.

Lande, R. 1977. The influence of the mating system on the maintenance of genetic variability in polygenic characters. Genetics 86:485-498.

Latter, B.D.H. 1965. The response to artificial selection due to autosomal genes of large effect. II. The effect of linkage on limits to selection in finite population. Aust. J. Biol. Sci. 18:1009-1023.

Latter, B.D.H. 1966. The interaction between effective population size and linkage intensity under artificial selection. Genet. Res. 7:313-323.

Lindgren, D. 1977. Genetic gain by progeny testing as a function of mating design and cost. In: Proc of the Third World Consultation on Tree Breeding. Session 6:Choosing Strategies for the Future, Canberra.

Lindgren, D., W.S. Libby, and F.L. Bondesson. 1989. Deployment to plantations of numbers and proportions of clones with special emphasis on maximizing gain at a constant diversity. Theor. Appl. Genet 77:825-831.

Lindgren, D., and A.C. Matheson. 1986. Increasing the genetic quality of seed from seed orchards by using the better clones in higher proportions. Silvae Genet 35:173-177.

MacNeil, M.D., D.D.Kress, A.E.Flower, R.P.Webb, and R.L.Blackwell. 1984. Effects of mating system in Japanese quail. Theor. Appl. Genet 67:403-406.

Madalena, F.E. and W.G. Hill. 1972. Population structure in artificial selection programs: simulation studies. Genet. Res. 20:75-101.

Namkoong, G. and J.H. Roberds. 1974. Choosing mating designs to efficiently estimate genetic variance components for trees. I. Sampling errors of standard analysis of variance estimators. Silvae Genetica 23:43-53.

Namkoong, G., E.B. Snyder, and R.W. Stonecypher. 1966. Heritability and gain concepts for evaluating breeding systems such as seedling seed orchards. Silvae Genetica 15:76-84.

Nasoetion, A.H., C.C. Cockerham, and D.F. Matzinger. 1967. Simultaneous selfing and partial diallel test crossing II. An evaluation of two methods of estimation of genetic and environmental variance. Biometrics 23:325-334.

Nicholas, F.W. and A. Robertson. 1980. The conflict between natural and artificial selection in finite populations. Theor. Appl. Genet. 56:57-64.

Payne, F. 1918. An experiment to test the nature of variation on which selection acts. Indiana Univ. Studies no. 36. Vol 5, pp. 3-45. Bloomington: Indiana University.

Pepper, W.D. 1983. Choosing plant-mating design allocations to estimate genetic variance components in the absence of prior knowledge of the relative magnitudes. Biometrics 39:511-521.

Pepper, W.D. and G. Namkoong. 1978. Comparing efficiency of balanced mating designs for progeny testing. Silvae Genetica 27:161-169.

Roberts, R.C. 1966a. The limits to artificial selection for body weight in the mouse. I. The limits attained in earlier experiments. Genet. Res. 8:347-360.

Roberts, R.C. 1966b. The limits to artificial selection for body weight in the mouse. II. The genetic nature of the limits. Genet. Res. 8:361-375.

Roberts, R.C. 1974. Selection limits in the mouse and their relevance to animal breeding. First World congress on Genetics Applied to Livestock Production. Vol. 1, pp. 493-509, Madrid.

Robertson, A. 1960. A theory of limits in artificial selection. Proc.Royal Soc.B 153:234-249

Robertson, A. 1961. Inbreeding in artificial selection programmes. Genet. Res. 2:189-194.

Robertson, A. 1970. A theory of limits in artificial selection with many linked loci. In, K. Kojima (ed.) Mathematical topics in population genetics, pp. 246-288. Springer-Verlag. New York.

Schuster, P. and K. Sigmund. 1989. Fixation probabilities for advantageous mutants: A note on multiplication and sampling. Mathematical Biosciences 95:37-51.

Sved, J.A. 1977. Opposition to artificial selection caused by natural selection at linked loci. In: Pollak, E., Kempthorne, O., and Bailey, T.B.(eds.), Proceedings of the International Conference on Quantitative Genetics, pp. 435-458. Ames, Iowa.

van Buijtenen, J.P. 1972. Efficiency of mating designs for second generation selection. P.103-126 in Proc. IUFRO Meet. on Progeny Testing.

Wright, S. 1977. Sewall Wright Vol. III: Experimental results and evolutionary deductions. Univ. of Chicago Press, Chicago and London. 613p.

Young, S.S.Y. and R.V. Skavaril. 1976. Computer simulation of within family selection in finite populations. Theor. Appl. Genet. 48:45-51

THE USE OF BEST LINEAR PREDICTION TO OBTAIN BREEDING VALUES FOR HEIGHT AND SURVIVAL IN 37 FULL-SIB PROGENY TESTS OF SHORTLEAF PINE (PINUS ECHINATA MILL.) ON THE OUACHITA AND OZARK-ST.FRANCIS NATIONAL FORESTS

T. La Farge and J. E. Gates [1]

U.S.D.A. Forest Service, Southern Region

Abstract.--Since 1978 the Tree Improvement Program of the Southern Region (Region 8) of the Forest Service has been progeny testing seed orchard clones of shortleaf pine. Breeding Population 1, located on the Ouachita and Ozark-St. Francis National Forests in Arkansas and Oklahoma, comprises 25 6 X 6 diallel crossing groups totalling 150 parents.

After 12 years of progeny test establishment, there is a great deal of imbalance in the data. Each year full-sib progeny tests were established at as many as five progeny test locations, but not all families could be included at each location, and most diallel crossing groups were less than complete. There were also several test failures due to summer droughts. Hence, the prospects of obtaining balanced ANOVAs and unbiased estimates of breeding values were formidable until the recent availability or Best Linear prediction (BLP) as a method of data analysis.

This paper reports predicted breeding values for height and survival of shortleaf pine in the National Forests in Arkansas and Oklahoma. It discusses advantages and disadvantages of BLP and some limitations encountered in applying this method by means of the Statistical Analysis System for Personal Computers (PC SAS).

Keywords: Pinus echinata Mill., Best Linear Prediction, full-sib, progeny test.

INTRODUCTION

In 1978 the Tree Improvement Program of the Southern Region (Region 8) of the Forest Service began establishing progeny tests of seed orchard clones in five species of the southern pines. Shortleaf pine (Pinus echinata Mill.) is an important species in the National Forest System, especially in the Piedmont and Mountain provinces. In the Region 8 Tree Improvement Program, Breeding Population 1, representing the Ouachita and Ozark-St. Francis National Forests in Arkansas and Oklahoma, is the largest of six breeding populations of

---

shortleaf pine. The mating scheme comprised full-sib crosses in 25 6 X 6 diallel crossing groups totalling 150 parents. All clones of this breeding population are located in the Ouachita Seed Orchard near Hot Springs, Arkansas.

Because of logistic problems in controlled breeding and several test failures resulting from summer droughts, there is a great deal of imbalance in the data available after 13 years of progeny test establishment. Each year full-sib progeny tests were established at as many as five progeny test locations, but not all families could be included at each location, and most diallel crossing groups were less than complete. Hence, the prospect of obtaining balanced ANOVAs and unbiased estimates of breeding values were formidable until the recent availability of Best Linear Prediction (BLP) as a method of data analysis (White et al. 1986; White and Hodge 1989).

Previously La Farge (1989) compared two methods of applying BLP. One method was designed for predicting breeding values for different target environments when genotype x environment interactions were involved or suspected. In the present case the simpler model is assumed, which is that there is only one breeding population, or zone, and there are no significant genotype x environment interactions.

METHODS

Progeny Test Design

The field designs of all progeny tests conformed to the standard randomized complete-block design used in the Region 8 Tree Improvement Program. In this design the tests are laid out in 10-tree row plots so that all plots within a block followed the same contour. Most tests contained five replicates, but three had only four. The 37 tests comprising this analysis were established in the eight years from 1978 through 1985. One test was established in 1978 and one in 1979. Five tests were planted in 1981, six in 1982, five in 1983, eleven in 1984, and eight in 1985.

The maximum number of tests in which any one crossing group was replicated in any one year was three, but in some years groups were included in only one or two tests. After 1978, all crossing groups were replicated in two years. This is the only breeding population in the Region 8 Program in which replication by years was attempted. However, in the present analysis year of establishment was not included as an effect.

Breeding Population 1 of shortleaf pine is subdivided into 25 crossing groups, of which 21 have been tested and measured so far. Each crossing group consists of six parents which are intercrossed to form a 6 x 6 element diallel. This design is a disconnected partial diallel, and all crossing groups are unrelated. Although the potential number of crosses in a 6 x 6 element diallel is 15, the largest number of crosses in any single crossing group in the current test is 14.

145

Site quality varied greatly, resulting in large differences in growth rate and survival among sites. Average test height at age 5 years ranged from 5.2 to 13.2 feet, and average percentage survival varied from 69.1 to 99.9.

The BLP Equation

All progeny tests included in this analysis were full-sib tests, and both the theory and methods of analysis used in the present investigation are described by White and Hodge (1989). A primary utility of BLP is that it can be used to predict parental genotypes in full-sib progeny tests.

A principal assumption of BLP is that the first and second moments are known (Henderson 1984, White and Hodge 1989). The second moments are specified in the C and V matrices. The C matrix, which is nonsymmetric, defines the genetic relationships between the observed full-sib family means at each site and the true yet unknown breeding values, g. Each column of C represents a parental breeding value to be predicted, and the elements comprising C are estimated from genetic theory.

The V matrix, which is symmetric, represents the variances and covariances between the observed phenotypic values. The main diagonal consists of variances of family means for each planting location. In full-sib progeny tests the covariances in the off-diagonals not equal to zero are covariances between family means which refer to either: (1) different tests with two common parents; (2) different tests with one common parent; or (3) the same test with one common parent (White and Hodge 1989). When these matrices have been constructed, the following formula can be used to predict the breeding values:

$$\hat{g} = C'V^{-1}y \tag{1}$$

where C and V are defined above, y = a vector of data representing observed deviations of the family means at each test location from the test location mean, and $\hat{g}$ = the breeding values to be predicted.

Procedures Used

All tests were measured at five years of age. The traits measured were height in feet and survival. The survival percentages were analyzed as the arcsin X the square root of percentage expressed in radians. In the Region 8 Program, diameter and straightness are not measured until the tenth year.

Space does not allow a complete discussion of the theory or procedures involved in BLP, but discussions of the theory and construction of C and V matrices are given by White et al. (1986) and White and Hodge (1989), and examples of operational C and V matrices are provided by La Farge (1989).

To generate the second moments needed to construct the C and V matrices, it is necessary to perform a combined ANOVA on the data. This was accomplished by means of the VARCOMP Procedure of the Statistical Analysis System for Personal Computers and and the MIVQUE0 Method (SAS 1987). The variance

components generated by Proc VARCOMP were then combined in appropriate equations to produce the second moments needed to construct the C and V matrices. The equations that are needed for this conversion are provided by White and Hodge (1989). The second moments were loaded into the C and V matrices by means of language provided in the SAS IML Guide for Personal Computers (1985).

The process of attempting to analyze such a large number of progeny tests with Proc VARCOMP presents data management problems on PC SAS. Proc VARCOMP generates an error reading when too much data are entered. Usually the largest data set that it will allow includes data from only five tests. Hence analysis of the data from 37 tests on PC SAS is out of the question. One solution is to divide the data into 5-test subsets and then obtain an average value for each variance component estimated (G. R. Hodge, personal communication).

The averaging of variance components was the method used. Although this method appears to have no theoretical basis, it seems to work. However, in the process of averaging variance component estimates, another problem arose. Which data sets should be averaged to make these estimates? In other Region 8 breeding populations, as many as four or five progeny tests are planted each year, and these tests comprise the same set of one or two crossing groups, including up to five check lots. However, as mentioned above, the tests in Shortleaf Breeding Population 1 were replicated by years as well as by sites or by locations. When these tests are grouped only by common years, many crosses and crossing groups are not common among tests. Such unmatched groupings tend to produce weak variance compents.

To compensate for this source of error, new groupings were assembled based on their common crossing groups. Six such groupings were found and were averaged to produce a set of variance components for each trait. One grouping was discarded for survival because the survival in the three tests comprising that grouping was so high that the variation was insufficient to produce strong variance components. The total subset of tests from which variance components were derived for height was 24; for survival the total was 21 tests. These variance components are listed in Table 1.

RESULTS

Breeding values for height and survival were predicted for a total of 123 parents, three of which were check lots common to most progeny tests. The breeding values of the best and worst of these parents and checks are given in Table 2 for height and survival. The breeding values for survival have been reconverted from radians to percentages.

Also included in Table 2 are the estimated correlations between the true and predicted genetic values (CRGG), which are obtained by means of the following equation:

$$CRGG = Corr(\hat{g}, g) = \{Var(\hat{g})/Var(g)\}^{1/2} \qquad (2)$$

(White and Hodge 1989), where $Var(\hat{g})$ is the variance of the predicted breeding values and $Var(g)$ is the variance of the true but unknown breeding values. Since best Linear Prediction provides no direct method of detecting significances of differences among treatment means, the estimation of a CRGG value for each breeding value is a useful if imperfect measure of the strength of the breeding value. Although there is no predetermined level of acceptability for values of CRGG, the closer each value is to unity, the better. Our experience with many breeding values suggest that they should not be less than 0.5.

Table 1. Variance component estimates based on averages of six test subsets for height and five test subsets for survival in 37 shortleaf pine progeny tests on the Ouachita and Ozark National Forests.

Source of variation	Variance component for:	
	Height	Survival
Var_F [1]	0.057200	0.0021490
Var_M	0.056577	0.0017991
Var_{FS}	0.013258	0.0022249
Var_{MS}	0.026943	0.0009755
Var_{FM}	0.045690	0.0016402
Var_{FMS}	0.017522	0.0056500
$Var_{FB(S)}$	0.012493	0.0012195
$Var_{MB(S)}$	0.003973	0.0008943
$Var_{FMB(S)}$	0.235810	0.0202050
Var_e	2.339910	0.3173500

[1] Var_F, Var_M, Var_{FS}, Var_{MS}, Var_{FM}, Var_{FMS}, $Var_{FB(S)}$, $Var_{MB(S)}$, $Var_{FMB(S)}$, and Var_e are, respectively, variances due to female, male, female X site, male X site, female X male, female X male X site, female X block-within-site, male X block-within-site, female X male X block-within-site, and tree-within-plot.

It is obvious from Table 2 that the breeding values predicted for height are stronger than those predicted for survival. The range of variation among predicted values for height is almost two feet, that for survival 10.9 percent. Further, the values for the CRGGs are generally much higher for height, although all values for survival are 0.57 or higher. Individual and family heritabilities, given in Table 3, also show the relative strengths of these statistics, as do the realized genetic gains in Table 4. The realized gains are calculated on the basis of the General Forest Area (G.F.A.) Mix. In the Region 8 Program G.F.A. check lots are usually represented by a sample of stand collections within a breeding zone.

Table 2. Rank comparisons of the best and worst shortleaf pine parents for height and survival in 37 progeny tests at age five on the Ouachita and Ozark National Forests.

Parental I.D.	Crossing group	Height	Height rank	CRGG [1] for height	Survival	Survival rank	CRGG for survival
		Feet			Percentage		
			BEST HEIGHT				
243	14	8.98	1	0.785	90.0	113	0.635
127	11	8.84	2	.774	91.8	89	.619
136	17	8.80	3	.819	94.1	37	.682
230	18	8.74	4	.784	93.6	44	.634
			BEST SURVIVAL				
332	18	8.15	48	.790	97.7	1	.638
104	9	8.41	18	.869	96.7	2	.753
142	7	8.24	39	.660	96.3	3	.660
150	22	8.16	47	.767	96.2	4	.603
			POOREST HEIGHT				
135	14	7.19	123	.875	88.2	122	.761
343	10	7.30	122	.767	91.6	91	.604
201	17	7.38	121	.873	90.3	108	.760
226	18	7.39	120	.829	91.6	95	.685
			POOREST SURVIVAL				
137	6	7.44	116	.798	86.8	123	.654
135	14	7.19	123	.875	88.2	122	.761
206	21	8.01	71	.760	88.5	121	.571
244	13	7.90	88	.758	88.7	120	.571
			CHECK LOTS				
S.P.A. Ozark [2]	51	7.62	108	.969	91.5	97	.920
G.F.A. Mix [3]	54	7.51	113	.977	89.3	116	.922
S.P.A. Ouachita	51	7.44	115	.974	93.1	62	.914

[1] The estimated correlations between true and predicted genetic values.
[2] Open-pollinated seed from a seed-production area.
[3] A mixture of seed collected from the general forest area.

DISCUSSION AND CONCLUSIONS

While the range of breeding values for each trait is not large, the differences for height seem to be great enough to achieve satisfactory gains. The percentage gain in height growth is in close agreement with those reported for other species with data combined from many tests, e.g. Kraus and La Farge (1982). The corresponding statistics for survival might be stronger if most of the progeny tests had had poorer survival than they did. Fortunately the poor survival in some tests is probably attributable to certain years with very dry summers. Because of the uncertainty of predicting years of severe summer drought or sites that may be prone to drought, selection for high survival or drought resistance probably has little practical value.

Table 3. Individual and family heritabilities for height and survival in 37 full-sib progeny tests of shortleaf pine on the Ouachita and Ozark National Forests.

	Heritability, h^2	
	Individual	Family
Height	0.08	0.96
Survival	0.02	0.53

Table 4. Realized genetic gains for height and survival in an unrogued shortleaf pine seed orchard based on 37 full-sib progeny tests in the Ouachita and Ozark National Forests.

Trait	Realized genetic gain
	Percentage
Height	7.52
Survival	3.96

The breeding values generated from these analyses actually have greater utility than merely for making backward selections and roguing the poorest parents. Although some roguing will be done, their primary purpose will be for use in making selections of the best trees in those families with the highest parental breeding values by means of the method discussed by Hodge et al. (1989). Also, since some of the 37 tests have reached age 10, diameter and straightness measurements will permit selection indexes for more traits to be utilized in the BLP procedures.

The primary utility of these methods is that they allow the combined analyses of data from many progeny tests over a period of time. Within limits BLP tolerates messy data, but poorly estimated breeding values occasionally need to be discarded. The CRGG values provide some guidance as to which breeding values are poorly estimated, and inspection of the data will usually indicate the reason for the low CRGG values (e.g., inadequate replication).

LITERATURE CITED

Henderson, C. R. 1984. Applications of linear models in animal breeding. University of Guelph, Guelph, Ontario, Canada. 462 p.

Hodge, G. R., G. L. Powell and T. L. White. 1989. Establishment of the second-generation selected population of slash pine in the Cooperative Forest Genetics Research Program. P. 68-74 in Proc. 20th Southern For. Tree Imp. Conf. Charleston, SC.

La Farge, T. 1989. Applications of best linear prediction to the analysis of five full-sib loblolly pine progeny tests. P. 315-322 in Proc. 20th Southern For. Tree Imp. Conf. Charleston, SC.

SAS Institute. 1985. SAS/IML guide for personal computers, version 6 edition. SAS Institute, Inc., Cary, North Carolina. 244 p.

SAS Institute. 1987. SAS/STAT guide for personal computers, version 6 edition. SAS Institute, Inc., Cary, North Carolina. 1028 p.

White, T. L., G. R. Hodge and M. A. Delorenzo. 1986. Best linear prediction of breeding values in forest tree improvement. P. 99-122 in Statistical Considerations in Genetic testing of Forest Trees, Proc. 1986 Workshop Southern Regional Information Exchange Group 40. Southern Coop. Series Bull. 324. Univ. of Florida, Gainesville, FL.

White, T. L. and G. R. Hodge. 1989. Predicting breeding values with applications in forest tree improvement. Kluwer Academic Publishers, Dordrecht, The Netherlands. 367 p.

FUSIFORM RUST INCIDENCE AND VOLUME GROWTH IN A FIRST-GENERATION BACKCROSS POPULATION, (SHORTLEAF X SLASH) X SLASH

C.D. Nelson[1]

Abstract.--The lack of fusiform rust resistance has restricted slash pine planting to sites with a low rust hazard. An interspecies backcross breeding study was undertaken to investigate the applicability of transferring the rust resistance of shortleaf pine into slash pine. Rust resistance and volume growth of a first-generation backcross population were evaluated after 7 years of field growth. The population consisted of 12 backcross families formed by mating 1 of 6 elite slash pine clones to 2 of 7 shortleaf X slash pine F1 hybrids. Rust incidence was moderate (25%), but differences among slash pine parents were highly significant (range 11% to 54%). Differences among F1 parents were not significant, however, 4 of the 12 backcross families were less rusted than the slash pine controls. Differences in tree volume among slash pine parents and among F1 parents were significant, and half the backcross families were larger in mean tree volume than the slash pine controls. From this test, no conclusions about the applicability of transferring rust resistance from shortleaf to slash pine with backcross breeding can be made, although the volume growth of slash pine was recovered in several backcross families. In future breeding cycles, multiple rust inoculation tests, coupled with early selection and accelerated breeding, will be required to conclusively evaluate this breeding method. In practice, the careful integration of early testing, accelerated breeding, and DNA marker-assisted selection may alleviate many of the traditional problems of backcross breeding in forest tree species.

Keywords: backcross breeding, interspecies breeding, fusiform rust resistance, marker-assisted selection.

INTRODUCTION

Interspecies backcross breeding is a method for transferring favorable alleles of a trait from one species (donor) to another (recipient). Usual requirements for employing this method include a zero or suboptimal level of trait expression in the recipient (and vice versa in the donor), qualitative inheritance of the trait, and a moderate degree of fertility between the two species. Within commercially important southern pines, these requirements appear to be present in several instances. For example, Brown (1964) recommended using the method to transfer genes for early height growth from slash pine (Pinus elliottii var. elliottii Englm.) to longleaf pine (P. palustris Mill.) as a means of eliminating the grass stage in longleaf. A second example, initiated by a USDA Southeastern Forest Experiment Station research unit, is aimed at transferring fusiform rust resistance from shortleaf pine (P. echinata Mill.) to loblolly pine (P. taeda L.) (Kraus 1986).

Slash pine is a host of the obligate biotrophic fungus, Cronartium quercuum (Berk.) Miyabe ex Shirai f. sp. fusiforme. The severity of the resulting fusiform rust disease has limited the planting of slash pine to sites with a low rust hazard. Currently, only intraspecies sources of resistance are available to slash pine breeders. This resistance is not well characterized and may be vulnerable to changes in the pathogen and environment (Snow et al. 1976). Alternative sources of resistance would greatly benefit the long-term breeding effort. Because it is apparently resistant to all cultures of fusiform rust (f. sp. fusiforme) (Kraus et al. 1982, Kraus and Powers 1984), shortleaf pine is a potential source. These factors, together with a knowledge of moderate interspecies fertility (Synder and Squillace 1966), suggest that a backcross breeding program may be an effective way to transfer rust resistance from shortleaf to slash pine.

---

[1] Research Geneticist, USDA Forest Service, Gulfport, Mississippi.

In a study reported by Wells et al. (1978), interspecies hybrids were made between shortleaf pine parents from 8 different geographic sources and a 21-tree slash pine pollen mix from north Florida. These hybrids and shortleaf and slash pine checks were tested at eight locations across the natural range of shortleaf pine. After 10 years, hybrids with north Georgia and southeast Arkansas parents were consistently taller than the other hybrids and the shortleaf pine checks. Rust incidence for all hybrids was well below (0% to 19%) the midparent level and in most cases nearly as low (0% to 6%) as the shortleaf pine checks. In the present study, hybrid trees with north Georgia and southeast Arkansas shortleaf pine parents were selected in a south Mississippi test planting and mated to elite slash pine clones. The resulting backcross progenies were grown in south Mississippi to test the shortleaf pine sources and the slash pine clones for their value as parents in a backcross breeding program and to provide a backcross population for further selection and breeding.

MATERIALS AND METHODS

F1 hybrid trees with north Georgia (Southwide Southern Pine Seed Source [SSPSS] 413) and southeast Arkansas (SSPSS 429) shortleaf pine maternal parents were phenotypically selected at age 16 years. The trees were growing in the south Mississippi test planting of a shortleaf X slash pine interspecies hybrid study (Wells et al. 1978). Slash pine parents had previously been progeny tested and selected for a fast growth rate by the Cooperative Forest Genetics Research Program (CFGRP). These clones also ranged from slightly below average to well above average for rust resistance. The original mating plan was to pollinate 8 to 10 hybrids from the 2 sources with an individual slash pine clone and then pool the seed by hybrid source. However, only one tree from source 429 and six from source 413 could be productively pollinated. As a result, the pollinations produced 12 backcross families (BC1)--6 single-tree test crosses for source 429 and 6 single crosses for source 413. Based on cone-collection data, these families consisted predominantly of full-siblings (>70%) and partially of paternal half-siblings (<30%).

Seedlings were grown in the Harrison Experimental Forest (Saucier, Mississippi) nursery in 1983 and planted at a field site near Lizana, Mississippi in January 1984. The field site was judged in the moderate-to-high category for fusiform rust hazard. A randomized complete block experimental design was used with 5 replications of 15-tree row plots. In addition to the 12 backcross families, 2 checks were included--slash pine and the F1 parents' open-pollinated progeny (F1-op). Two plots of each check were planted in each replication. The source of the slash pine check was not documented, but it is thought to be a bulk of open-pollinated seed collections from 6 to 10 resistant slash pine trees[2]. The F1-op seeds were bulked by source before growing in the nursery and then planted in the field plots by source. A border row of slash pine, presumably from same source as the check, was placed completely around the planting. Also in January 1984, an industrial slash pine plantation was established adjacent to all sides of the test planting. A 30-ft-wide firebreak was maintained between the test planting and the industrial plantation.

Data were collected on each test tree for 1-year survival, 2-year height; and 7-year total height, stem diameter at 1 ft, and rust incidence. Heights and diameters were measured to the nearest 0.1 ft and 0.1 in, respectively. Assuming a cylindrical base (length of 1.0 ft) and a parabaloid top, stem volume (cu ft) was computed for each tree. Rust incidence was scored on a 0 to 3 scale as follows: 0 = no gall, 1 = branch gall(s) only, 2 = branch-into-stem gall(s) and branch gall(s) or no gall, and 3 = stem gall(s) and branch-into-stem gall(s) or branch gall(s) or no gall. Rust score was used to create two binomial variables--gall and stem gall: If rust score > 0, then gall = 1, otherwise gall = 0; and if rust score > 1, then stem gall = 1, otherwise stem gall = 0. Mortality was also analyzed as a binomial variable: If the tree was alive at planting and dead at age 7 then mortality = 1, otherwise mortality = 0. In the surrounding industrial slash pine plantation, rust incidence was scored on 15 randomly selected 15-tree linear plots.

---

[2] This information was kindly provided by John Pait of the Container Corporation of America, Callahan, Florida.

The data were analyzed using the GLM procedure of SAS (SAS Institute 1985) and the following model: Y = M + R + E + RE + W, where M is the mean, R is replication, E is entry (i.e., families and checks), RE is replication X entry interaction, and W is the within-plot error. All effects were assumed fixed. Replication 5 was heterogeneous in terms of height growth and ground vegetation so it was omitted before the analysis. The entry sums of squares were partitioned into 5 degrees of freedom (df) for males, 1 df for female sources, and 5 df for male X female source interaction. In addition, three linear contrasts were constructed to test for differences between each check and the backcrosses and between the checks. Means of males, females, backcross families, and checks were compared with t-tests at a type I error rate of 0.05.

RESULTS

Means for survival, growth, and rust incidence in the test planting are presented in Table 1. Field observations suggested a possible class of stunted trees. They were not easily scored during data collection but were apparent in a frequency histogram of the 7-year height data. The histogram was strongly skewed to the left with stunted trees ranging from 2.5 to 6.9 ft. Large stem galls appeared to stunt some trees, but in general no reason for stunting was apparent. Thus, all trees less than 7.0 ft were omitted from the growth analysis, but not from the rust or mortality analyses. The percentage of trees under 7.0 ft was not associated with any class of progeny or individual family. Rust incidence in the 225-tree sample of the industrial slash pine plantation was 39.6% for all galls and 30.7% for stem galls.

Table 1. Summary of survival, growth, and fusiform rust incidence in a (shortleaf X slash) X slash test planting near Lizana, Mississippi.

Variable	Age	Mean	Std. Dev.	Min.	Max.
Survival (%)	1	85.6			
	2	80.5			
	7	74.9			
Growth					
Height (ft)	2	2.58	0.95	0.5	6.5
	7	16.95	3.91	7.0	27.2
Diameter (in)[a]	7	3.81	1.02	0.9	7.1
Volume (cu ft)	7	0.83	0.52	0.02	3.87
Rust					
Stem gall (%)	7	20.5	40.4	0	1
Gall (%)	7	25.3	43.5	0	1

[a] stem diameter at 1 ft.

GLM (type III) results for the 7-year mortality, growth, and rust data are summarized in Table 2. Before GLM analyses, stem volume was transformed to the square root scale to remove positive skewness from the data. For growth traits, replication and entry effects were significant, while only entry effects were significant for mortality and rust incidence traits. Males were highly significant for all traits, and female sources were significant for height, volume, and mortality and nonsignificant for diameter and all rust traits. Male X female source interaction was highly significant for rust traits, but nonsignificant for growth traits and mortality. Linear contrasts comparing the checks and checks versus backcrosses were nonsignificant for all growth traits and mortality. The slash versus backcrosses and slash versus F1-op contrasts were significant for the rust traits, with slash significantly less infected than backcrosses or F1-op.

Table 2. F-test results from GLM analyses of 7-year mortality, stem growth, and fusiform rust incidence data.

Source	df	Mort.	Growth			Rust	
			Ht.	Dia.	Vol.[a]	Stem gall	Gall
Rep.	3	ns	**	**	**	ns	ns
Entry	14	**	**	**	**	**	**
Male	5	**	**	**	**	**	**
Female[b]	1	*	*	ns	*	ns	ns
Male X Female[b]	5	ns	ns	ns	ns	**	**
Slash vs. F1	1	ns	ns	ns	ns	**	*
Slash vs. BC	1	ns	ns	ns	ns	**	**
F1 vs. BC	1	ns	ns	ns	ns	ns	ns
Rep. X Entry	42	ns	**	ns	ns	ns	ns
Within-plot							
Mortality	900						
Growth	633						
Rust	668						

[a] Square root of stem volume.
[b] Source of shortleaf in hybrid female: north GA vs. southeast AR (see text for details).
Notes: ns = not significant at p<.05.
    * = significant at p<.05.
    ** = significant at p<.01.

Table 3 presents the results from the mean separation tests for mortality, volume, and rust incidence. T-tests grouped the males into two groups of three trees for both stem volume and percentage galled (gall*100). The grouping is rather distinct for percentage galled and nondistinct for volume. CFGRP breeding value data for the male clones showed two of the Coop's most resistant clones (males 1 and 4) to be included in the low-incidence group, along with an average clone (male 5). The clones grouped in the high-incidence group ranked intermediate (R40=29) to average (R40=43) in breeding value[3]. A comparison of volume rankings showed little or no correlation with the Coop's breeding values. Differences between female sources were significant for volume growth, with the north Georgia source producing larger backcross and F1-op progenies.

The significant male X female source interaction for rust incidence is clearly evident in Table 3. For percentage galled, males 3 and 2 interact strongly with the two female sources. Male X female source interaction is essentially absent for mortality and volume. T-tests on entry means for percentage galled show six families equal in rust incidence to the slash checks and nine equal or better than the F1-op (Table 4). Entry means for percentage stem galled are also shown in Table 4. Few entry rank-order changes were noted, and each was contained within the upper or lower halves of each ranking (i.e., 2 to 5 and 9 to 12).

[3] R40 is a predicted breeding value for percentage of rust incidence, scaled to an environment in which nonselected slash pine is 40% infected.

Table 3. T-test comparisons of males and female sources for 7-year mortality, stem volume, and percentage galled.

Mortality (%)

| | Males | | | | | | | |
Females	1	3	5	4	2	6	mean[a]	wind
429 (AR)	13	20	17	22	22	30	21a	33
413 (GA)	13	18	23	30	38	40	27b	30
Slash								26
Mean[a]	13a	19ab	20abc	26abc	30bc	35c		

Volume (sq. rt.)

| | Males | | | | | | | |
Females	1	4	3	5	6	2	mean[a]	wind
413 (GA)	0.96	0.92	0.91	0.89	0.81	0.76	0.88a	0.91
429 (AR)	0.95	0.89	0.85	0.74	0.75	0.77	0.83b	0.82
Slash								0.89
Mean[a]	0.95a	0.91a	0.88ab	0.81bc	0.78c	0.76c		
Coop rank	6	3	4	1	5	2		
Coop VOL	0.18	1.13	1.08	2.60	0.26	1.34		

Gall (%)

| | Males | | | | | | | |
Females	5	1	4	6	3	2	mean[a]	wind
413 (GA)	9	8	19	39	31	78	28a	19
429 (AR)	12	15	11	31	58	30	26a	34
Slash								12
Mean[a]	10a	11a	14a	35b	44bc	52c		
Coop rank	4	1	2	6	5	3		
Coop R40	36	7	15	43	37	29		

[a] Means followed by same letter are not significantly different (p<.05).
Notes: Coop rank is CFGRP rank of breeding values for 15-year volume and 5-year rust incidence. Low ranks indicate high volume and low rust.
Coop VOL is 15-year stem volume breeding value in cu ft deviations.
Coop R40 is 0.80 * R50 (i.e., scaling R50 to a 40% rust environment).

DISCUSSION

Various positive and negative aspects of this study must be accounted for in the interpretation of the results. On the positive side, several points can be noted, such as a moderate, well-dispersed incidence of rust, reasonably good survival and growth in four of five replications, no replication or replication X entry effects for mortality or rust incidence, highly significant male effects for all traits, and significant male X female effects for rust incidence. Negative points include one test environment, mixed mating design, lack of necessary checks (i.e., susceptible slash and shortleaf), and lack of rust data between planting and age 7 years. In general, the scope of any inferences drawn should be limited to the tested environment, including inoculum source, site conditions and climate, and to the tested parental population.

Table 4. T-test comparisons of backcross families and checks for 7-year percentage galled and backcross family and check means for percentage stem galled.

Female[a]	Male	Gall (%)[b]	Stem Gall (%)
413	1	8a	4
413	5	9a	7
429	4	11ab	6
429	5	12ab	10
Slash	FL wind	12ab	7
429	1	15abc	13
413	4	19abc	16
F1	MS wind	27bcd	23
429	2	30cd	28
413	3	31cd	24
429	6	31cd	29
413	6	39d	28
429	3	58e	42
413	2	78f	73

[a] Each 413 is a different hybrid tree with north GA shortleaf, while each 429 is the same hybrid tree with southeast AR shortleaf (see text for details).
[b] Means followed by the same letter are not significantly different (p<.05).

The geographic source of shortleaf pine in the hybrid parents had a significant effect on 7-year mortality and growth, but not on rust incidence. Backcross progenies with north Georgia shortleaf pine germplasm (source 413) were larger in stem volume than those of southeast Arkansas (source 429), but they also suffered greater mortality. The larger volumes were mostly due to increased height, as source 413 backcrosses averaged 17.3 ft versus 16.6 ft for source 429. Difference in stem diameter was not significant--3.87 inches versus 3.70 inches for sources 413 and 429, respectively. Although statistically significant for volume and mortality, the applicability of these results is limited because of the small genetic sample sizes from the two sources--six parents from source 413 and only one from source 429.

Rust incidence was primarily conditioned by the slash pine parents, and very little improvement over resistant slash was observed in the backcrosses per se. However, the incidence level in the backcross progeny of one slash clone (male 5) was markedly improved compared with its CFGRP resistance breeding value. Unfortunately, this clone's backcross progeny showed a substantial decrease in growth relative to its breeding value for volume growth. The performance of the backcross progenies of clone 1 is also worth noting. CFGRP breeding values ranked clone 1 first of the six tested in resistance and sixth in growth, yet its backcross progeny performance ranked first in growth and second in rust resistance.

The results of this study appear to be consistent with those for rust incidence in an artificial inoculation test of (shortleaf X loblolly) X loblolly backcrosses conducted by Kraus et al. (1982). In their study, backcrosses with parents of unknown resistance were not significantly less infected than nonselected loblolly pine. In contrast, La Farge and Kraus (1980) and Kraus (1986) found (shortleaf X loblolly) X loblolly backcrosses per se to be significantly less infected than loblolly pine, and in Kraus (1986) these backcrosses were not significantly more infected than shortleaf pine. As in the shortleaf-loblolly pine studies, the potential for completely recovering the height growth of the faster growing species seems promising in the BC1 generation. Thus, in theory, clonal propagules of fast-growing, resistant BC1 individuals would constitute a desirable clonal variety of 3/4 slash:1/4 shortleaf pine. In practice, however, clonal testing would be required to ensure superior growth and resistance performances of the selected

BC1 clones. Seed-propagated hybrid varieties, such as F1-op, may have some potential (Hyun 1974); however, careful testing and parental selection would also be required to ensure consistent, superior performance.

True backcross varieties, propagated by seed or as clones, normally require several additional generations of selection and, ideally, would retain resistance genes only from shortleaf pine. The likelihood of obtaining quality parental material in a BC4 or BC5 is small due to the time required to advance a population of forest trees to this stage. However, with the help of DNA marker-assisted selection and accelerated breeding, it is likely that both the number of generations and the time to achieve one generation will be substantially reduced.

Currently, two forms of marker-assisted selection should be applicable to southern pine backcross breeding. Genomic selection (Hillel et al. 1990) uses species-specific DNA fingerprints to estimate the proportion of nuclear DNA in backcross individuals. Presuming that a qualitatively inherited trait is under selection, individuals expressing this trait are first selected, and then their DNA fingerprint is analyzed and used to select those most similar to the recurrent parent (or dissimilar to the donor). Alternatively, the DNA is first analyzed and then only trees with desirable DNA fingerprints are tested for trait expression. In either case, such selections in the BC1 are likely to contain 5% to 10% more recurrent DNA than expected. Hillel's formulae suggest that genomic selection in species such as the southern pines will identify BC2 individuals equivalent in recurrent parent DNA to a BC4 generation produced without the assistance of DNA markers.

A second DNA marker-assisted approach requires considerably more molecular genetic development and screening work but promises much greater precision. A saturated linkage map and a mapped trait are required. This approach allows marker-based selection for recurrent DNA, as in genomic selection, but additionally, it provides a means for selecting against donor DNA in the region of the selected trait loci (Young and Tanksley 1989). This added selection opportunity is very beneficial, because donor DNA adjacent to the trait loci is very difficult to select against in the absence of linked markers (Hanson 1959). Obviously with accelerated breeding, the time for each generation is greatly reduced and the gain in time is multiplicative --the reduced number of generations X the reduced number of years per generation. This kind of time savings should increase the likelihood of successfully implementing a multigenerational backcross breeding program.

Research efforts in recurrent selection and interspecies or intraspecies breeding for fusiform rust resistance should focus on the genetic mechanisms conditioning the interaction of the host and pathogen. Although backcrosses per se offered no improvement over resistant slash pine in this study, pathogen populations may possibly adapt to the resistance factor(s) present in the slash pine. Breeders must be concerned with the rate of this adaptation, which depends to a large extent on the genetics of the host and the pathogen. Interorganismal genetic studies have shown that incompatible interactions (i.e., "resistance") may be conditioned by only one gene in the host and one in the pathogen, while several loci in each may be prevented from expression by an epistatic type of gene action (Loegering 1978). A change from avirulence to virulence at one pathogen locus would spread rapidly in the pathogen population by rendering the corresponding resistance gene in the host ineffective. In the future, breeders will likely require a diverse collection of resistance factors to maintain genetic protection from fusiform rust. Thus, incorporating and maintaining shortleaf pine resistance factors in slash pine genetic backgrounds will be a useful, if not necessary, breeding activity.

In summary, interspecies backcross breeding may prove useful in the southern pines, but several pieces of knowledge and technology, including a rigorous understanding of the inheritance of important traits, the development and practical application of DNA marker systems, and a continuing commitment to accelerated, multigenerational breeding, must be integrated.

LITERATURE CITED

Brown,C.L. 1964. The seedling habit of longleaf pine. Georgia Forest Research Council and University of Georgia. Athens. 68 pp.

Hanson,W.D. 1959. Early generation analysis of lengths of heterozygous chromosome segments around a locus held heterozygous with backcrossing or selfing. Genet. 44:833-837.

Hillel,J., T.Schaap, A.Haberfeld, A.J.Jeffreys, Y.Plotzky, A.Cahaner, and U.Lavi. 1990. DNA fingerprints applied to gene introgression in breeding programs. Genet. 124:783-789.

Hyun,S.K. 1974. The possibility of F2 utilization of Pinus rigida X taeda. Korean J. Breed. 6(2):123-133.

Kraus,J.F. 1986. Breeding shortleaf X loblolly pine hybrids for the development of fusiform rust-resistant loblolly pine. South. J. Appl. For. 10:195-197.

Kraus,J.F., and H.R.Powers, Jr. 1984. Susceptibility of shortleaf pine seedlings to infection by Cronartium quercuum f. sp. fusiforme. Plant Dis. 68(4):324-325.

Kraus,J.F., H.R.Powers, Jr., and G.Snow. 1982. Infection of shortleaf X loblolly pine hybrids inoculated with Cronartium quercuum f. sp. echinatae and C. quercuum f. sp. fusiforme. Phytopath. 72(4):431-433.

La Farge,T., and J.H.Kraus. 1980. A progeny test of (shortleaf X loblolly) X loblolly hybrids to produce rapidly growing hybrids resistant to fusiform rust. Silvae Genet. 29(5-6):197-200.

Loegering,W.Q. 1978. Current concepts in interorganismal genetics. Ann. Rev. Phytopath. 16:309-320.

SAS Institute Inc. 1985. SAS user's guide: statistics. Version 5 edition. Cary, NC: SAS Institute Inc. 956 pp.

Snow,G.A., R.J.Dinus, and C.H.Walkinshaw. 1976. Increase in virulence of Cronartium fusiforme on resistant slash pine. Phytopath. 66(4):511-513.

Synder,E.B., and A.E.Squillace. 1966. Cone and seed yields from controlled breeding of southern pines. USDA For. Serv. Res. Pap. SO-22. 7 pp.

Wells,O.O, P.E.Barnett, H.J.Derr, D.T.Funk, T.La Farge, E.R.Lawson, and S.Little. 1978. Shortleaf X slash pine hybrids outperform parents in parts of the Southeast. South. J. Appl. For. 1:28-32.

Young,N.D., and S.D.Tanksley. 1989. RFLP analysis of the size of chromosomal segments retained around the Tm-2 locus of tomato during backcross breeding. Theor. Appl. Genet. 77:353-359.

THE VERIFICATION, DESCRIPTION AND INHERITANCE PATTERNS OF PUTATIVE
P. virginiana x P. clausa and P. clausa x P. virginiana
HYBRID PINES

J. D. Porterfield[1]

Abstract.--A study examining reciprocal interspecific pine control crosses of sand pine (Pinus clausa (Chapm. ex Engelm.) Vasey ex Sarg.) and Virginia pine (Pinus virginiana Mill.) was undertaken with the primary objectives of verifying individual F_1 hybrids and describing each of the crosses. Secondary objectives included determining the mode of inheritance of individual traits as to whether intermediate or dominant in both crosses and determining whether there are significant reciprocal hybrid differences for these traits. Thirty-three different morphological, anatomical, biochemical and phenological characters were finally chosen for evaluation and description of parental species and hybrids. Putative hybrids and their parental species were analyzed by canonical discriminant analyses and a comparison of trait means. Several good discriminating characters were found between the parental species and putative hybrids. Pollen shedding stage was by far the best and is recommended for future interspecific pine hybrid verification studies.

Reciprocal crosses between sand pine and Virginia pine were verified, but contamination during pollination was suggested by a few putative F_1 hybrids which closely resembled the female parent in each cross. These crosses indicate a very close taxonomic relationship between these species. Only four of 18 useful traits (parental species significantly different at $P < .05$) measured were intermediate for the sand by Virginia hybrids and only 62 percent were intermediate for Virginia by sand hybrids. Significant reciprocal hybrid differences were evident in 16 percent of 32 traits measured for P. virginiana x P. clausa and P. clausa x P. virginiana. The hybrids of Virginia with sand pine were healthier and better adapted to the site at Tillery, N.C. than sand pine and performed as well if not better than Virginia pine based on observations of survival, height and diameter. This indicates that this hybrid might be useful on other sites in the southeastern United States.

Keywords: Pinus clausa (Chapm. ex Engelm.) Vasey ex Sarg.) Pinus virginiana Mill., interspecific hybridization, pine hybrid verification, reciprocal crosses, dominance, P. clausa x P. virginiana.

CLONAL PROPAGATION AND GENETIC TESTING OF VIRGINIA PINE

J. Aimers-Halliday, C.R. McKinley and R.J. Newton[1]

Abstract.—Texas Christmas tree growers plant approximately 800,000 Virginia pine (Pinus virginiana Mill.) seedlings each year. In 1981 a tree improvement cooperative was formed with the objective of providing genetically improved planting material to these growers. Clonal propagation could potentially be an integral component of this and other tree improvement programs through production of limited and/or proven genotypes. One technique of clonal propagation is tissue culture of cotyledon explants and the subsequent production of plantlets for operational plantings. Before this system of propagating Virginia pine can be considered successful, it is imperative that micropropagated plantlets are evaluated in field trials. Three trials consisting of both plantlets and seedlings were established in spring, 1990, to compare the performance of plantlets to genetically improved seedlings of similar genetic background. This is the first phase of our Virginia pine clonal field testing program. After one growing season, plantlets were shorter and had a slightly lower survival rate. Plantlets were smaller and more variable in size and age when planted compared to seedlings, due to the constraints of the tissue culture system.

Keywords: Pinus virginiana Mill., in vitro propagation, clonal field trials, plantlet.

INTRODUCTION

The estimated market for Christmas trees in Texas is over 3 million trees annually. Imports from the northern and western United States have, to date, captured most of this market (Chandler 1985). However, the Christmas tree industry has been steadily growing in Texas. In recent years, approximately 800,000 Virginia pine seedlings have been planted annually by Texas Christmas tree growers. In 1990, approximately 400,000 Texas-grown Christmas trees were sold, the large majority of which were Virginia pine. The value of the harvest was approximately $8.0 million, with a total economic impact of at least $17 million (J.W. Chandler, pers. comm. 1991).

In 1981 a cooperative of the Texas Christmas Tree Growers Association, the Texas Agricultural Extension Service, and the Texas Forest Service was initiated. The objective of the cooperative is to provide genetically improved planting

---

stock for Christmas tree growers via the Virginia pine Christmas tree improvement program (McKinley 1989). In 1983 a seed orchard of approximately 4 ha was established at Magnolia Springs, Texas, with 50 families. Following evaluations from genetic tests, the poorest 25 families were removed leaving the best 600 trees for seed production (McKinley 1989). The first cone crop was collected at Magnolia Springs in 1987 and the first genetically improved Virginia pine seedlings became available in the fall of 1988. It is anticipated that the seed orchard will be in full production by 1995 and will produce approximately 50 kg of seed per year (C.R. McKinley, pers. comm. 1991).

Vegetative propagation could potentially be a valuable component of the Virginia pine tree improvement program. Some of the potential benefits are: the capturing of non-additive genetic variation and thus increased genetic gain (McKeand 1981, Libby and Rauter 1984, McKeand and Weir 1984, Ahuja and Muhs 1985, Johnson 1988); the reduction in the lag between selection and reforestation with select individuals (McKeand 1981); more information regarding genetic parameters (Libby 1969, Burdon and Shelbourne 1974); amplification of control-pollinated seed; in vitro selection (Mott and Amerson 1984); and propagation of transgenic plants (van Buijtenen and Lowe 1989).

Clonal propagation can easily be integrated into a classical tree improvement program. However, it is important to visualize the clonal option as a technique to maximize genetic gain at any point in time, rather than genetic improvement per se (Barnes and Burley 1987). It is vitally important to maintain genetic variability in broadly-based breeding populations. The rapid progress made in mass clonal propagation techniques can be applied to the conservation of this genetic variability and also allow for rapid exploitation of superior recombinants in each generation of the breeding program (McKeand 1981, Barnes and Burley 1987, Shelbourne 1988). In vitro propagation systems appear to have the greatest potential for mass propagation (McKeand 1981, van Buijtenen and Lowe 1989).

There are three main approaches for in vitro culture of plants:
(1)   Production of adventitious shoot buds directly from excised plant parts or from callus, then induction of rooting.
(2)   Induced proliferation of the shoot apex, axillary or fascicular buds to produce multiple shoots which can then be rooted.
(3)   Production of callus and suspension cultures from unorganized tissue explants, and then induction of somatic embryogenesis.

The most successful micropropagation systems to date for gymnosperms have involved induction of adventitious buds on explants of embryos or young seedlings (David 1982). Chang et al. (1991) successfully achieved organogenesis in cotyledon explants of Virginia pine. Approximately 5000 plantlets have recently been produced in our lab via this procedure.

Achieving the potential genetic gains from vegetative propagation depends entirely on the good performance of clonal material in the field (McKeand 1981). Information from clonal field trials indicates that vegetative propagules may initially grow significantly slower than seedlings (McKeand and Frampton 1984, Horgan 1987), have similar growth rates (Menzies and Klomp 1988), or grow faster (Bennett et al. 1986). Vegetative propagules sometimes show desirable "mature"

characteristics, such as resistance to rust (McKeand and Frampton 1984) and better form (McKeand 1985, Bennett et al. 1986, Menzies and Klomp 1988). Conversely, vegetative propagules have shown undesirable "mature" characteristics such as poor rooting, plagiotrophic growth, and decreased growth and survival in field plantings (McKeand 1985, St Clair et al. 1985, Ritchie and Long 1986).

The objective of this paper is to report on the first phase of our Virginia pine clonal propagation and genetic testing program. Height growth and survival of plantlets and seedlings from similar genetic sources are compared after one year of growth in the field.

METHODS

Plantlet and Seedling Propagation

Open-pollinated seed from families 1-38 and 1-78 were obtained from the Virginia pine tree improvement program. Some of the seed was cold stratified and sown for containerized seedling production. Approximately 300 seed were retained for containerized plantlet production via methods developed by Chang et al. (1991) using embryonic cotyledon explants. After 4 weeks of acclimation in plastic tents, the plantlets were kept in the same greenhouse as the seedlings until field planting. Only the largest plantlets were selected for field planting.

Field Trial Design

Field trials for testing plantlet versus seedling performance were established in spring 1990. Three locations were chosen, as sites representative of Virginia pine Christmas tree growers land – in central Texas, southeast Texas and northeast Texas. The field trial design was a randomized block design with five blocks and 15 four-tree plots per block. Ten of these plots within each block were containerized seedlings (twenty seedlings each from Virginia pine families 1-78 and 1-38) and five of the plots per block were plantlets from families 1-78 and 1-38. Because of the small number of ramets per ortet, no within clone analyses was attempted.

Height and survival were measured monthly. The plantlets and seedlings are managed – in terms of weed control, clipping and shearing, and irrigation where needed – by the landowners, with the understanding that all the test trees are treated equally.

Statistical Analyses

Covariance analyses were performed using the general linear model (GLM) procedure in the Statistical Analysis System (SAS Institute, Cary, NC), with initial height used as the covariate. Data from each location was analyzed separately. Raw means for initial and final heights, and the least square means (adjusted for initial height) were determined using the MEANS and LSMEANS SAS procedures.

RESULTS

The covariance analyses are summarized in Table 1. Initial height is the only main effect showing a high level of significance (1%) all locations. Plant type was significant at the 10% level at the central Texas location, at the 1% level at the southeast location, and at the 5% level at the northeast location. The plant-type by initial-height interaction was significant (5% level) at the southeast Texas location only.

Table 1. Summary of the covariance analyses for Virginia pine plantlet and seedling heights after one growing season in the field, initial height being the covariate.

			LOCATION				
		CENTRAL TEXAS		SOUTH-EAST TEXAS		NORTH-EAST TEXAS	
		Sum of		Sum of		Sum of	
SOURCE	d.f	Squares	F	Squares	F	Squares	F
REPLICATION	4	522.23	3.90***	2565.62	18.62***	312.61	1.12
PLANT TYPE (T)	1	110.17	3.29*	321.45	9.33***	461.78	6.63**
SOURCE (S)	1	11.10	0.33	7.04	0.20	11.42	0.16
INITIAL HEIGHT (I)	1	2731.77	81.59***	2636.73	76.53***	2646.57	37.98***
T * S	1	41.48	1.24	24.94	0.72	158.96	2.28
I * T	1	72.22	2.16	143.00	4.15**	143.47	2.06
I * S	1	26.74	0.80	12.21	0.35	0.23	0.00
ERROR	276	17154.00		9302.53		19231.10	

* Indicates significance at 0.10 level of probability
** Indicates significance at 0.05 level of probability
*** Indicates significance at 0.01 level of probability

At the end of their first growing season in the field, the plantlets were significantly shorter than the seedlings, but this was partly due to their lower initial height. Table 2 summarizes the raw means and least square means for plantlet and seedling height, respectively. The least square means have been adjusted for the variation due to initial heights. These adjusted means are graphically represented in Figure 1.

Percent survival for both plant types was good at all locations, ranging from 88% to 99% (Figure 2) and with a mean survival of 92% for the plantlets and 97% for the seedlings. At the central and northeast Texas locations seedling survival was better compared with plantlets. Considerable stress, in terms of drought at the central location and flooding at the northeast location, were encountered soon after planting. In contrast, plantlet survival was comparable or better at the southeast Texas location where stress occurred later in the season with little rainfall in September and October.

Table 2. Raw means and least square means for plantlet and seedling heights

| LOCATION | PLANT TYPE | SOURCE | RAW MEANS | | ADJUSTED MEANS | |
			Initial Height (cm)	Final Height (cm)	Final Height (cm)	Std. Error
CENTRAL TEXAS	Plantlet	1-38	13.4	28.6	27.2	0.84
	Plantlet	1-78	9.5	24.3	27.4	1.44
	Seedling	1-38	15.4	32.6	29.1	0.71
	Seedling	1-78	13.2	28.3	27.2	0.69
S.E. TEXAS	Plantlet	1-38	11.0	28.3	29.8	0.81
	Plantlet	1-78	7.6	25.4	30.1	1.42
	Seedling	1-38	11.3	31.9	32.9	0.70
	Seedling	1-78	11.5	32.0	32.7	0.72
N.E. TEXAS	Plantlet	1-38	10.1	30.1	32.5	0.85
	Plantlet	1-78	10.8	31.0	32.7	1.36
	Seedling	1-38	13.0	39.4	38.7	0.60
	Seedling	1-78	12.3	35.2	35.1	0.96
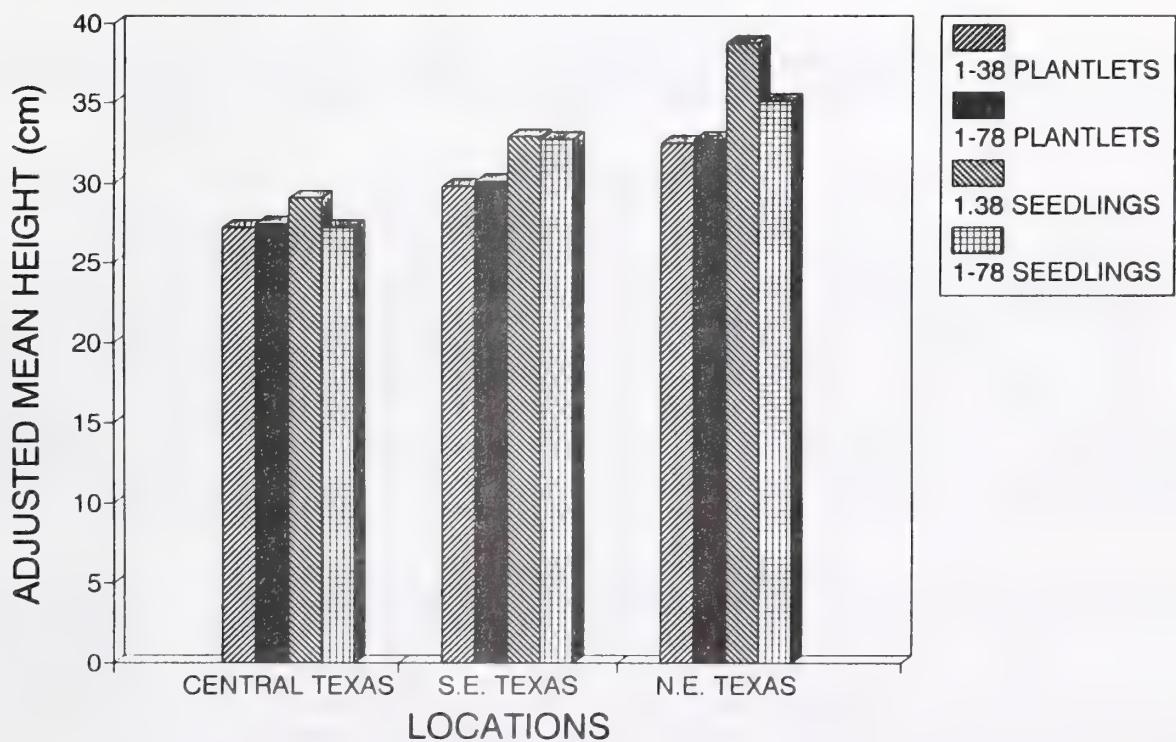
Figure 1. Least square means for height of plantlets and seedlings from two genetic sources after one growing season at three different field locations. The means are adjusted for initial height.

165
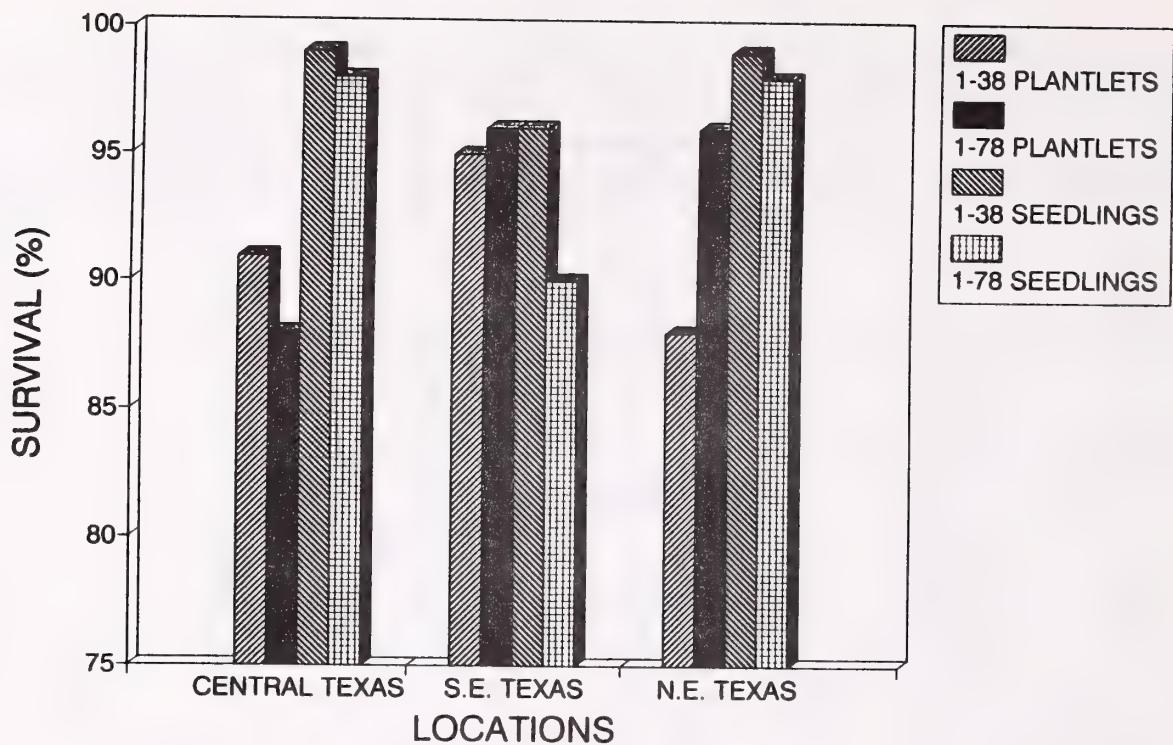
Figure 2. Percent survival of plantlets and seedlings from two different genetic sources after one growing season at three different field locations.

DISCUSSION

This paper presents the initial results on the relative performance of plantlets and seedlings from our first phase of clonal field trials. After one year in the field, plantlets have a 5% lower survival rate and were significantly shorter compared with seedlings. This has been reported in other similar studies, but plantlets showed more comparable growth in subsequent years (McKeand and Frampton 1984, McKeand 1985, Ritchie and Long 1986). In our study, the plantlets may have been disadvantaged because they were significantly smaller than the seedlings when planted. It is difficult to produce planting stock via different methods and have them uniform in size at planting.

At the end of 1991, after two years in the field, the plantlets and seedlings will be ranked for form. Height and diameter measurements will be taken until the test trees are harvested as Christmas trees beginning in November 1992. In this study, both plantlets and seedlings were container grown in a greenhouse. Because of the strong preference for nursery grown bare-root planting stock, a pilot scheme with 300 plantlets was established at a commercial nursery in March 1991. The plantlets are being managed similar to bare-root planting stock.

The second phase of our clonal field trials was begun in the spring of 1991, with the establishment of clonal uniformity and clonal stability trials. The plantlets were derived in the same manner as the plantlets from the first phase of trials reported here. There is also strong interest in cloning Christmas trees

of sufficient age and size to indicate their superiority. We are currently developing a micropropagation procedure using fascicular bud proliferation techniques.

LITERATURE CITED

Ahuja,M.R. and H.J.Muhs. 1985. *In vitro* techniques in clonal propagation of forest tree species. Advanced Agricultural Biotechnology 14:41–49.

Barnes,R.D. and J.Burley. 1987. Vegetative propagation for improved tropical forest trees. *In* Abbott,A.J. and R.K.Atkin (Eds.) Improving Vegetative Propagated Crops. Academic Press, London. Chapter 12 (pp 211–227).

Bennett,I.J., C.M.Tonkin, M.M.Worth, E.M.Davison, and J.A.McComb. 1986. A comparison of growth of seedling and micropropagated *Eucalyptus marginata* (Jarrah). I. Early growth to 2 years. Forest Ecology and Management 14:1–12.

Bornman,C.H. 1984. Application of *in vitro* culture technology in clonal forestry. *In* International Symposium of Recent Advances in Forest Biotechnology. June 10–13, 1984, Grand Traverse Resort Village, Traverse City, Michigan. Pp 178–194.

Burdon,R.D. and C.J.A.Shelbourne. 1974. The use of vegetative propagules for obtaining genetic information. New Zealand Journal of Forest Science 4:418–425.

Chandler,J.W. 1985. Growing Christmas trees in Texas. Texas Agricultural Extension Service. Texas A&M University System. College Station, TX 77843. L–1733 4 pp.

Chang,S., S.Sen, C.R.McKinley, J. Aimers–Halliday, and R.J.Newton 1991. Clonal propagation on Virginia pine (*Pinus virginiana* Mill.) by organogenesis. Plant Cell Reports, in press.

David,A. 1982. *In vitro* propagation of gymnosperms. *In* Bonga,J.M. and D.J.Durzan (Eds.) Tissue Culture in Forestry. Martinus Nijhoff/Dr. W.Junk Publishers, The Hague. Pp 72–108.

Horgan,K. 1987. *Pinus radiata*. *In* Bonga,J.M. and D.J.Durzan (Eds.) Tissue Culture in Forestry. Volume 3. Martinus Nijhoff Publishers, Dordrecht, Netherlands. Pp 128–145.

Johnson,G.R. 1988. A look to the future: Clonal forestry. *In* Menzies,M.I.; J.P.Aimers; and L.J.Whitehouse (Eds.) Workshop on Growing Radiata Pine Cuttings, May 1986. Ministry of Forestry, FRI Bulletin No. 135. Pp. 79–85.

Libby,W.J. 1969. Some possibilities of the clone in forest genetics research. *In* Bogart,R. (Ed.) Genetic lectures. Volume 1. Oregon State University Press. Pp 121–136.

Libby,W.J. and R.M.Rauter. 1984. Advantages of clonal forestry. The Forestry Chronicle 6:145–149.

McKeand,S.E. 1981. Loblolly pine tissue culture: Future uses in southern forestry. Technical Report No. 64. School of Forest Resources, North Carolina State University, Raleigh, North Carolina. 50p.

McKeand,S.E. 1985. Expression of mature characteristics by tissue culture plantlets derived from embryos of loblolly pine. Journal of the American Society for Horticultural Science 110(5):619–623.

McKeand,S.E. and L.J.Frampton Jr. 1984. Performance of tissue culture plantlets of loblolly pine *in vivo*. *In* International Symposium of Recent Advances in Forest Biotechnology, June 1984, Traverse City, Michigan. Pp 82–91.

McKinley,C.R. 1989. The Virginia pine Christmas tree improvement program. Texas Forests News 67(4)15–18.

Menzies,M.I. and B.K.Klomp. 1988. Effects of parent age on growth and form of cuttings, and comparison with seedlings. *In* Menzies,M.I.; J.P.Aimers; and L.J.Whitehouse (Eds.) Workshop on Growing Radiata Pine Cuttings, May 1986. Ministry of Forestry, FRI Bulletin No. 135. Pp. 18–40.

Mott,R.L. and H.V.Amerson. 1984. Role of tissue culture in loblolly pine improvement. *In* International Symposium of Recent Advances in Forest Biotechnology, June 1984, Traverse City, Michigan. Pp. 24–36.

Ritchie,G.A. and A.J.Long. 1986. Field performance of micropropagated Douglas fir. New Zealand Journal of Forestry Science 16(3):343–356.

Shelbourne,C.J.A. 1988. The role of cuttings in the genetic improvement of forest trees. *In* Menzies, M.I., J.P.Aimers and L.J.Whitehouse. Workshop on Growing Radiata Pine Cuttings, May 1986. Ministry of Forestry, FRI Bulletin No. 135. Pp. 7–14.

St Clair,J.B., J.Kleinschmit and J.Svolba. 1985. Juvenility and serial vegetative propagation of Norway spruce clones (*Picea abies*). Silvae Genetica 34(1):42–48

van Buijtenen,J.P. and W.J.Lowe. 1989. Incorporation of biotechnology into tree improvement programs. Proceedings of the 20th Southern Forest Tree Improvement Conference, Charleston, South Carolina, June 26–30. Pp. 60–67.

STERILIZATION AND GERMINATION PROCESSES FOR IMPROVING MICROPROPAGATION EFFICIENCY OF THREE SOUTHERN PINES

A.M. Diner[1]

Abstract. In vitro adventitious bud initiation from newly germinated seedlings of loblolly, slash, and longleaf pine was studied to compare the effects of varying decontamination procedures and germination media. Seeds of loblolly and slash pines were either cold-stratified 1 month in moist peat moss, or immersed several days in 1% hydrogen peroxide at 29°C with seed coat micropyles perforated. Stratified, rinsed seeds were decontaminated in 30% hydrogen peroxide, then germinated on water-agar. Longleaf pine seed was germinated either by several days' seed immersion in 1% hydrogen peroxide, or by incubation of 30% hydrogen peroxide-sterilized seed on water-agar. Seedlings were germinated on agar at 20°, 25°, and 29°C. All aseptic seedlings of the three species were incubated intact on adventitious bud initiation medium for 2 days. Seedlings were then cut to cotyledons and hypocotyls for 12 days' additional incubation on initiation medium.

Decontamination procedures were almost completely effective. Growth of endogenous microbial contaminants was infrequent. Hydrogen peroxide-germinated seedlings were small, chlorotic and poorly developed. Micropropagation efficiency of each species was significantly higher using seedlings germinated on water-agar than in hydrogen peroxide.

Keywords: Pinus taeda, Pinus elliotti, Pinus palustris, tissue culture, in vitro

INTRODUCTION

Efficient in vitro micropropagation of conifers is hampered by several factors, possibly the most common of which is failure or inability to decontaminate the starting tissues completely. Numerous chemicals and processes have been employed for surface-sterilization of seed coats or decoated seedlings at least partially enclosed by gametophyte. Most of these processes include use of aqueous hypochlorite at solute concentrations ranging from 7.5% weight per volume (w/v) (von Arnold and Eriksson 1985) to 0.3% (Cheng and Voqui 1977). Though micropropagation success has been reported for numerous conifer species (Newton et al. 1989), decontamination efficiency relative to either pregerminated or germinated seeds has not been addressed, in spite of its pertinence to the costly, labor-intensive sequence of steps necessary to micropropagation, or to the limited availability of select seed lots.

[1] Plant physiologist, USDA Forest Service, Southern Forest Experiment Station, Alabama A&M University, Normal, AL 35762.

Seed dormancy in some species imposes an additional obstacle to immediate and direct use of seeds for micropropagation. While dormancy may be overcome by stratification, the use of a hydrogen peroxide seed soak has come into frequent use for rapid, high-frequency, relatively synchronous germination (Mott and Amerson 1981, Diner and Mott 1982, Newton et al. 1989). However, in preliminary tests at the same temperatures, hydrogen peroxide-germinated seedlings of loblolly (<u>Pinus</u> <u>taeda</u> L.), and slash (<u>P</u>. <u>elliotti</u> Engelm. var. <u>elliotti</u>) pines were chlorotic, smaller, and less well developed than those that had been first stratified, then germinated aseptically on water-agar.

The objectives of this study were (1) to develop protocols for decontamination of loblolly, slash, and longleaf (<u>P</u>. <u>palustris</u> L.) seed and germinated seedlings, and (2) to compare the micropropagation efficiency (ramets/genotype) of tissues from seedlings either germinated in hydrogen peroxide or stratified and then germinated on agar.

METHODS

Seed used in this study was obtained from International Forest Seed Co., Odenville, Alabama, and included loblolly pine seedlot RS22032 from Livingston Parish, Louisiana, slash pine seedlot IF BP-5 from south Georgia, and longleaf pine seedlot BC 12003 from Bay County, Florida. For germination of cold-stratified loblolly and slash pine, seeds were first bagged in cheesecloth and immersed in $4^{\circ}C$ water for 24 hr. The water was then replaced with water-saturated peat moss, in which the bagged seeds were maintained at $4^{\circ}C$ for 1 mo. Rinsed, stratified seeds were immersed 30 min in 30% aqueous hydrogen peroxide, rinsed three times in sterile distilled water, and placed on 1.6% w/v water-agar in petri dishes. Dishes with seeds were sealed with Parafilm and incubated under laboratory ambient illumination ($50uE/m^2/sec$) at 20°, 25°, or $29^{\circ}C$ until germination terminated. Germinated seeds were decoated. Seedlings enclosed in gametophytes were agitated 15 to 20 min in approximately 100 ml 50% aqueous household bleach (5.25% hypochlorite) with a drop of Tween 20, then rinsed three times in sterile distilled water.

For germination of loblolly and slash pine in hydrogen peroxide, seed micropyles were perforated and seeds were immersed in 1% aqueous hydrogen peroxide at $29^{\circ}C$. The solution was replaced daily for 5 to 6 days. Germinated seeds were decoated and surface-sterilized in dilute bleach as described above.

Longleaf pine seeds were germinated either by immersion in 1% hydrogen peroxide as described above, or by placing decontaminated seeds on water-agar for several days. Decontamination was effected either by two successive 30-min soaks in 30% hydrogen peroxide separated by 5 to 6 hrs at room temperature, or by 45 to 60 min agitation in 50 to 100% household bleach to which 1 drop of Tween 20 per 100 ml disinfectant had been added. Rinsed seeds were then germinated at 20°, 25°, or $29^{\circ}C$ on 1.6% water-agar in petri dishes as described above for loblolly and slash pines. Adventitious bud frequency differences by seed germination treatment and by species were tested using Chi-square.

In preliminary experiments, the asynchronous germination of all three species invariably led to seedlings of different ages in the germination medium, regardless of the method used. However, newly germinated seedlings of longleaf pine showed very early reduction in sensitivity to cytokinin, similar to that reported for <u>P</u>. <u>radiata</u> D. Don (Aitken-Christie et al. 1985). Therefore,

seedlings with roots extruded no more than 3 mm for all three species were used in this study. Seedlings were aseptically extracted from gametophytes of the three species and applied flat to 0.8% agar-solidified Brown and Lawrence medium (Brown and Lawrence 1968) modified to contain 1.46 gram per liter (g/l) glutamine as the sole source of amino nitrogen (BLG medium) and supplemented with 10 milligrams per liter (mg/l) 6-benzyladenine (BA) in petri dishes. Dishes sealed with Parafilm were incubated 2 days at 25°C under ambient laboratory illumination for growth and detection of any microbial contamination. Cotyledons and hypocotyls of aseptic seedlings were separated, and placed with hypocotyl apices inverted on medium of the same composition for an additional 12 days. Green, swollen tissues were then transferred to a Gresshoff and Doy half-strength (GD/2) medium (Mehra-Palta et al. 1978) with 10 g/l each of sucrose and activated charcoal added. Cotyledons and hypocotyls of any genotype showing collective failure to enlarge on the BA-supplemented medium, as well as those developing an overall reddening or browning, were discarded. Subsequent biweekly tissue transfers were to Litvay's medium (Litvay et al. 1981) with 1.46 g/l glutamine (LMG) and 10 g/l sucrose. The pH of all media was adjusted to 5.5 before autoclaving. Water-agar and all solid media were gelled with 8.0 g/l agar (Sigma Chemical Co., St. Louis, MO). Standard incubation conditions were 20°C, and 16-hr days of 250 uE/m^2/sec cool-white illumination for all stages of in vitro micropropagation following the above-described initial 2 days' treatment of intact seedlings at 25°C. Adventitious bud numbers were determined between 8 and 12 weeks from seed excision. Genotypes discarded prior to transfer to GD/2 medium were not included in the data.

RESULTS AND DISCUSSION

Seed of all three species germinated faster (3 to 5 days) and more synchronously at 29°C either in hydrogen peroxide or on water-agar than they did on water-agar at the lower temperatures of 25°C (4 to 9 days) or 20°C (5 to 14 days). Although endogenous contaminant growth was infrequent to negligible, the large amounts of condensation that accumulated on the inner suface of petri dish lids at 29°C tended to drip and spread any incidental contaminant growth. The moderate amount of condensation that accumulated at 25°C did not preclude selection of that temperature for further studies.

It should be mentioned that preliminary experiments to decontaminate seedlings had followed the suggestions of other workers (Crump et al. 1989), that seedlings extracted from their gametophytes be immersed for periods as short as 1 min in very dilute disinfectants. These included 0.01% household bleach or 0.01% aqueous sodium-o-phenylphenate tetrahydrate with 1 drop of Tween 20 per 100 ml disinfectant, or 0.001% Physan 20 (Maryl Products, Tustin, CA). These treatments produced no immediately noticeable tissue damage, but resulted in inevitable necrosis of at least the abaxial surface of cotyledons. Thus, cotyledons intact to their respective hypocotyls began to reflex rearward within 2 days on BLG medium. Cotyledons so affected commonly became tightly coiled during subsequent days when individually placed on BLG medium. Apparently, tissues at other surfaces of the cotyledons, protected in the closed needle cluster during disinfection, remained viable and thus responsive to the cell-stimulatory effects of BA, resulting in expansion of only those tissues.

Seeds of all three species germinated in 1% hydrogen peroxide were consistently chlorotic and less well developed than those germinated on water-agar at the same temperature and for similar periods of time. Tissues were soft and spongy and difficult to manipulate without damage manifested later by necrotic zones. Longleaf pine seedlings expressed these characteristics to a greater degree than did seedlings of either loblolly or slash pine. Longleaf pine hypocotyls showed little elongation in 1% hydrogen peroxide; roots remained extremely short though seeds were allowed to remain in the solution for several days under germination conditions. Germination of the three species on water-agar ultimately resulted in an overall average of 28.1 adventitious buds, compared with 11.5 for germination in hydrogen peroxide. These differences were highly significant ($p < 0.01$) with a Chi-square value of 7.5. The average number of ramets per clone for each species was 21.0 for loblolly, 14.1 for slash, and 20.5 for longleaf pine. The species differences were not significant ($p > 0.05$). Germination on water-agar more than doubled the final clone sizes in all three species relative to germination in hydrogen peroxide, as shown in Table 1. Maximum adventitious bud frequencies from hydrogen peroxide-germinated loblolly and slash pine were 111 and 46, respectively; those from stratified seedlings were 132 and 71, respectively. Hydrogen peroxide-germinated longleaf pine generated a maximum of 28 buds, while tissues of seedlings germinated on water-agar showed 102.

Table 1. Micropropagation efficiency in loblolly, slash and longleaf pine seedlings germinated in 1% hydrogen peroxide versus an agar substrate.

	Micropropagation Efficiency					
	------------- Species--------------					
	loblolly		slash		longleaf	
	x^1/n^2	Max.	x/n	Max.	x/n	Max.
Germination Medium:						
1% H_2O_2	13.6/67	111	9.0/35	46	11.0/40	28
Water-Agar	30.0/55	132	21.0/35	63	33.0/31	102

[1] Average ramets/genotype
[2] Number of genotypes tested

LITERATURE CITED

Aitken-Christie, J., A.P. Singh, K.J. Horgan, and T.A. Thorpe. 1985. Explant developmental state and shoot formation in _Pinus radiata_ cotyledons. Bot. Gaz. 146: 196-203.

Brown, C.L., and R.H. Lawrence. 1968. Culture of pine callus on a defined medium. For. Sci. 14: 62-64.

Cheng, T.Y., and T.H. Voqui. 1977. Regeneration of Douglas fir plantlets through tissue culture. Science 198: 306-307.

Crump, L., S. Solomon, S. Thames, and C. Rhyne. 1989. An in vitro propagation protocol for the longleaf pine. Jour. Miss. Acad. Sci. 42.

Diner, A.M., and R.L. Mott. 1982. Direct inoculation of five-needle pines with Cronartium ribicola in axenic culture. Phytopathology. 72: 1181-1184.

Litvay, J.D., M.A. Johnson, D. Verma, D. Einspahr, and K. Weyrauch. 1981. Conifer suspension culture medium development using analytical data from developing seeds. Inst. Paper. Chem. Tech. Paper Series No. 115, 17 p.

Mehra-Palta, A., R.L. Mott, and R.H. Smeltzer. 1978. Hormonal control of induced organogenesis. Experiments with excised plant parts of loblolly pine. Tappi 61: 37-40.

Mott, R.L., and H.V. Amerson. 1981. A tissue culture process for the clonal production of loblolly pine plantlets. N.C. Agric. Res. Serv. Tech. Bul. No. 271.

Newton, R.J., S. Sen, F. Fong, and P. Neuman. 1989. Enhancement of shoot organogenesis in conifers. Pp. 168-175 in Proc. 20th Southern For. Tree Imp. Conf. Charleston, SC.

von Arnold, S. and T. Eriksson. 1985. Initial stages in the course of adventitious bud formation on embryos of Picea abies. Physiol. Plant. 64: 41-47.

A TISSUE CULTURE SYSTEM FOR MATURE TREES USING SECONDARY WOOD GROWTH FOR EXPLANT MATERIAL

E. N. Hiatt and R. M. Allen[1]

Abstract.--Regeneration of southern pines through tissue culture has proven difficult when using explant material from adult trees. One of the major stumbling blocks in successfully culturing long-lived woody species is rejuvenation. Ontogenetically young secondary wood growth taken near the base of the tree will potentially display juvenile-like culture responses. A sterilized increment borer was used to collect samples of secondary wood growth from trees at least 40 years in age. Samples were taken from four southern pine species: longleaf pine (Pinus palustris Mill.), slash pine (P. elliottii Englem.), loblolly pine (P. taeda L.), and shortleaf pine (P. echinata Mill.). Longleaf and slash pines were more amenable to culture than loblolly and shortleaf pines. Increment cores were sectioned into disks which were placed in liquid medium. After 6 weeks, disks were transferred to solid medium. A high percentage of disks developed callus. In slash and longleaf pines, cultures were produced which grew well and could be maintained for many subcultures. Some of these cultures exhibited chloroplast development, when moved to light. In some cases, the green callus developed an embryogenic appearance.

Keywords: Pinus palustris, P. elliottii, P. taeda, P. echinata, xylem parenchyma, resin ducts.

INTRODUCTION

Strong economic and scientific reasons exist for cloning individual mature forest trees that have demonstrated their superiority over time. However, vegetative propagation with mature conifers has not been economically feasible. A callus system of regeneration should be able to produce a large number of plantlets with little explant material, but regeneration from conifer callus is difficult. Although, there are many cases of success when starting with immature or juvenile tissue, there has been much less success in producing shoots or somatic embryos from callus of mature conifers. Gladfelter and Phillips (1987) report a low frequency of shoot bud formation in mature-tree callus of Pinus eldarica.

Rejuvenation presents a difficult obstacle to regenerating mature woody plants. Tissues that have undergone phase change are not easily converted back to juvenile characteristics (Wareing, 1987). A plausible approach is to use explant material that has not gone through phase change. Hackett (1985) states, "juvenile characteristics such as rooting potential may be preserved at the base

---

[1] Agricultural Science Associate II and Professor, Department of Forest Resources, Clemson University, Clemson, South Carolina.

of plants in ontogenetically young tissues (meristems), while maturation occurs in the periphery of the plant in ontogenetically older but chronologically young tissues." We have based our regeneration studies on explants from the base section of mature southern pine trees, endeavoring to take advantage of ontogenetically less mature tissues. We reasoned that it may be advantageous to start the callus cultures from xylem parenchyma of the inner sapwood which would be ontogenetically much younger than cambium cells at the same tree height.

The ability of ray parenchyma to generate callus tissue in response to wounding has long been known. If bark is removed but the tangentially exposed xylem is kept moist by promptly covering it with foil or plastic film the wound will soon become covered with a wound callus that originates primarily from the ray cells (Zimmerman and Brown, 1971). In studying stress induced cleavages in early wood, Amos (1953) found that the resulting cavities filled with callus in Pinus radiata, whose ray parenchyma are bounded by primary wall, but no callus was formed in the cavities of Psuedotsuga menziesii or Picea glauca whose ray parenchyma become thickened with secondary wall soon after formation. Harris and Barnett (1975) found examples of differentiated callus nodules in cavities of felled Pinus ponderosa. They attributed wood ray cells as the initiation source for this callus.

Xylem parenchyma cells can be cultured. Barker (1953) cultured 50-year-old cells from the medullary sheath region of Tilia americana. Successful conifer callus cultures arising from the resin ducts were obtained from Pinus radiata (Pardos, 1976) and from Pinus sylvestris (Kondrasheva, 1973; Kondrasheva and Yatsenko-Khmelevsky, 1974). White (1967) produced callus cultures from resin ducts "deep in the wood" of 20- to 60-year-old trees of Picea glauca. However, Zimmerman and Brown (1971) were unsuccessful in getting proliferation of the older ray cells in the xylem of 1- and 2-year-old twigs of Pinus elliotti and Pinus palustris.

This work reports the culturing of xylem parenchyma from the sapwood of mature trees of longleaf pine (Pinus palustris Mill.), slash pine (P. elliottii Englem.), loblolly pine (P. taeda L.), and shortleaf pine (P. echinata Mill.).

MATERIALS AND METHODS

Explant Selection

Samples were taken from 4 loblolly pines, 10 slash pines, 21 longleaf pines, and 3 shortleaf pines. Trees were located on the Clemson Experimental Forest, Clemson, South Carolina and were 40-60 years of age, with sapwood ranging from 30-40 rings in thickness. Only trees judged to be of superior vigor and form were used for explant material. After initial studies, longleaf and slash pines were found to be more amenable to culture than loblolly or shortleaf pines, so further studies concentrated on longleaf pine and slash pine.

175

Explant Collection

Samples of secondary wood growth were taken from older trees with increment borers. The increment borers were separated into their individual parts, wrapped in aluminum foil, and autoclaved prior to use in the field. To remove resin, increment borers were cleaned with 95% ethanol after each use. The tree bark was chopped away from the desired spot, exposing approximately 5 cm^2 of xylem, and the spot was well rinsed with 95% ethanol. A 5 mm diameter core was taken at stump height (approximately 30 cm from ground). Samples were immediately returned to the lab within the borer, where they were removed under a laminar flow hood. Despite the rather crude method of explant collection, no surface sterilization was necessary. Pardos (1976) and White (1967) used either disinfectants or flaming to surface sterilize their explant material. A number of the cores, however, were flamed in initial tests to ensure that callus growth was not originating from cambium cells which might have contaminated the core during sample collection. No callus growth could be attributed to cambium contamination, so flaming was discontinued. The increment cores, approximately 20 cm long, were sliced into 1-2 mm cross sections (disks). The cambium and the heartwood, generally, were excluded and depending upon the age of the parent tree, wood from 3-40 years in age was used.

Cultures were initiated at all times of the year, with some individual trees being sampled at several different times. When multiple samples were taken from the same tree, the cores were taken to one side and slightly above or below previous sample sites. The lower portion of trees sampled in warm weather was sprayed with benzene hexachloride solution as protection against bark beetles.

Supplemental tests were done using wood growth taken from roots of longleaf pine. The soil was removed from around the taproot and a lateral root. The outer covering was cut away and the surface rinsed with 95% ethanol. Sterile increment borers were used to take samples from within the roots. Roots were cultured in the same manner as secondary wood growth taken from the trunk.

Culturing Method

Six to eight wood disks were placed in 50 ml Erlenmeyer flasks containing 15 ml liquid medium. Foam stoppers were used to allow for gas exchange. Flasks were kept in a Lab-line Environ-Shaker at 25°C, in the dark, shaking at 100rpm. Liquid DCR medium (Gupta and Durzan, 1985) was used at pH 6.0 (pH adjusted using either 2N KOH or .15N HCl). Various amounts and combinations of the following growth hormones were used: 6-benzylaminopurine (BAP); α-naphthaleneacetic acid (NAA); and 2,4-dichlorophenoxyacetic acid (2,4-D). DCR medium was prepared at 3% sucrose. Every two weeks the liquid medium was removed, using a sterile pasteur pipet attached to a vacuum line, and replaced with fresh medium. After six weeks the disks were moved to agar (0.6%) solidified plates, and remained in the dark for varying periods of time before being moved to shelves with fluorescent or a combination of fluorescent and GE Gro&Sho plant lights. Cultures were exposed to 16 hours of light and 8 hours of dark. Once on solid medium, cultures were transferred approximately every four weeks. Small (60 X 15 mm) petri dishes were used. Plates were not wrapped with Parafilm^R, but they were kept in clear polycarbonate boxes (Petawawa Germination Boxes,

Spencer-Lemaire Industries Limited, Alberta, Canada) to reduce medium dehydration and to reduce contamination.

<div align="center">RESULTS</div>

Liquid Culture

Studies were conducted using longleaf pine and slash pine to find the optimum period of time required in liquid medium before transfer to solid medium. The medium used for this study was DCR 1 mg/L BAP 2 mg/L NAA (DCR1BAP2NAA). Cultures were transferred to solid medium, at two week intervals, from four weeks to ten weeks after initiation. Disks were also placed immediately upon solid DCR1BAP2NAA plates. Six weeks was chosen as optimum for liquid culture, because cultures exhibited no further visible callus development after six weeks. Less than 5% of the disks without a liquid medium treatment developed callus. In other studies, more than 70% of the disks in cultures of loblolly, slash, and longleaf pines, initially maintained in liquid medium, exhibited some degree of callus development. Approximately 50% of the cultures of shortleaf pine displayed callus development.

Callus Growth

In studies with loblolly pine and shortleaf pine, cultures grew little before callus darkened and growth ceased. Our work has concentrated on longleaf and slash pines, and all the results that follow are from studies performed with these two species. Studies with longleaf pine or slash pine produced callus that could be maintained through many subcultures. Callus cultures on solid medium had better growth if they remained in the dark for several weeks after placement on solid medium. Callus cultures in the dark were mostly opaque with some browning. After cultures were removed to lighted shelves, some of the cultures exhibited a green color. Microscopic examination of the callus confirmed that a portion of the cells had developed chloroplasts. In most cases the green callus existed in conglomeration with dark or opaque callus and increased slowly. Some green callus took on an embryogenic appearance, but no nodules, shoots, etc. were observed.

Callus was obtained from disks taken along the entire width of the sapwood. Disks taken from the heartwood did not show callus development. No difference was found in the percentage of cultures from the inner sapwood versus the outer sapwood that developed chloroplasts.

The source of the callus appeared to be the epithelial cells lining the resin ducts. Callus grew first and most from vertical resin ducts which were sliced along the face of the disks. Callus also grew from the ends of horizontal resin ducts and the ends of nonsliced vertical ducts (Figure 1). There was no evidence of uniserate rays developing callus. Pardos (1976) working with Pinus pinaster and White (1967) working with Picea glauca both denoted resin ducts as the source of callus growth in the xylem. Our research provided no grounds to refute this premise.

Figure 1. Longleaf pine disk with callus growth. The disk was cultured for 6 weeks in liquid DCR 1 mg/L BAP 2 mg/L NAA and one week on solid DCR .5 mg/L BAP .05 mg/L NAA, in the dark at 25°C. The tree was 56 years old and the disk was taken from the inner sapwood.

Longleaf Pine Study

Increment cores were taken from seven longleaf pine trees, in a 55-year-old plantation. One increment core was taken from each tree and 36 disks were sectioned from each core. Twelve disks were placed in each of the following media: DCR 1 mg/L BAP 2 mg/L NAA; DCR 1 mg/L BAP 2 mg/L 2,4-D; or DCR 2 mg/L 2,4-D. Cores were transferred to the same medium solidified with 0.6% agar after six weeks in liquid medium. Seventy-six percent of the all disks developed callus in the six weeks while in liquid medium. The differences in the percentage of disks that produced callus were attributed to genetic variation as well as varying responses to media type (Table 1). After seven weeks on solid medium, cultures were moved to lighted shelves (fluorescent lights only). Six weeks after being moved to light, 23% of all cultures had some degree of chloroplast development.

Root Study

Two increment cores were taken above ground (from stump height and ground level) and 2 were taken below ground (from the taproot and a lateral root). Two 56-year-old longleaf pines were utilized for this study. The increment cores were sectioned into 48 disks. Disks were cultured in DCR medium supplemented either with 2 mg/L 2,4-D alone; 2 mg/L 2,4-D plus 1 mg/L BAP; or 3 mg/L BAP alone. Cultures in DCR 3mg/L BAP showed no growth in liquid medium and were discarded from the test. All further results ignore the disks placed in DCR 3 mg/L BAP. After 6 weeks in liquid, disks were transferred to the same medium type plus 0.6% agar. Longleaf pine, 07, had 91% of disks develop callus and

tree 15 had 90% of disks develop callus. The percentage of disks that produced callus from each explant source can be seen in Table 2. Cultures on solid medium were kept in the dark for 11 weeks. Four weeks after cultures were moved to lighted shelves (fluorescent and Gro&Sho plant lights combined) the following percentage of cultures had developed chloroplasts. By explant source: stump height, 57%; ground level, 55%; taproot, 33%; and lateral root, 57%.

TABLE 1. Callus development on longleaf pine disks after 6 weeks in liquid medium.

Tree	DCR Basal Medium plus		
	1 mg/L BAP 2 mg/L NAA	1 mg/L BAP 2 mg/L 2,4-D	------ 2 mg/L 2,4-D
	--------------- Disks with callus (percents) ---------------		
07	83	92	100
08	83	33	8
09	100	58	83
10	50	42	42
11	100	92	100
12	100	100	100
13	83	75	83

Table 2. Callus development on disks of longleaf pines (07 & 15) taken from the following tree positions; stump height, ground level, taproot and lateral root. Disks maintained for 6 weeks in liquid culture followed by 3 weeks on solid medium (9 weeks).

Tree Position	DCR Basal Medium plus			
	2 mg/L 2,4-D		2 mg/L 2,4-D 1 mg/L BAP	
	6 weeks	9 weeks	6 weeks	9 weeks
	------------ Disks with callus (percents) ------------			
Stump height	83	96	76	100
Ground level	67	79	75	83
Taproot	100	100	100	100
Lateral root	92	100	56	72

DISCUSSION

Callus cultures which continued to increase through many subcultures were derived from xylem parenchyma located in secondary wood growth. Although cells seemed to lose their ability to divide and dedifferentiate once in heartwood, cells the entire width of sapwood can produce viable callus. Cells which are decades old remain vital. These viable cells may be in the minority of all cells present in wood, but enough cells are living to produce callus. Callus derived from these approximately 30-year-old, nonphotosynthetic cells had the ability to produce chloroplasts, indicating that the cells of origin maintain proplastids.

Some cultures produced green callus with an embryogenic appearance. We feel that the potential for obtaining plantlets from these calluses is present, but that the right set of culture conditions has yet to be found. Mature trees are recalcitrant in culture, making regeneration difficult. The parenchyma cells lining the resin duct may be in a position in the tree which is chronologically old, and at the same time ontogenetically young. These cells may retain a high enough degree of juvenility to make them more amenable to in vitro regeneration.

LITERATURE CITED

Amos, G. L. 1953. Radial fissures in early wood of conifers. Aust. J. Bot. 2:22-34.

Barker, W. G. 1953. Proliferative capacity of the medullary sheath region in the stem of _Tilia americana_. Am. J. Bot. 40:773-778.

Gladfelter, H. J. and G. C. Phillips. 1987. De novo shoot organogenesis of _Pinus eldarica_ Medw. in vitro. I. Reproducible regeneration from long-term callus cultures. Plant Cell Rep. 6:163-166.

Gupta, P. K. and D. J. Durzan. 1985. Shoot multiplication from mature trees of Douglas-fir (_Pseudotsuga menziesii_) and sugar pine (_Pinus lambertiana_). Plant Cell Rep. 4:177-179.

Hackett, W. P. 1985. Juvenility, maturation and rejuvenation in woody plants. Hort. Rev. 7:109-155.

Harris, J. M. and J. R. Barnett. 1975. Differentiated callus nodules in resin pockets of _Pinus ponderosa_ Laws. N. Z. J. For. Sci. 5:226-229.

Kondrasheva, N. Yu. 1973. Comparative capacity for proliferation of resin-duct cells of branches and stem sapwood of _Pinus sylvestris_ in tissue culture. Lesnoi Zhurnal. 16:31-33.

Kondrasheva, N. Yu. and A. A. Yatsenko-Khmelevsky. 1974. The ability of Scots pine resin ducts to form callus in artificial culture. Byulleten' Moskovskogo Obshchestva Ispytatelei Prirody (Otdel Biologicheskii). 79:132-139.

Pardos Carrion, J. A. 1976. Cultivation in vitro of xylem explants taken from the stem of <u>Pinus</u> <u>pinaster</u> trees, with special emphasis on the behavior of resin canals. Anales del Instituto Nacional de Investigaciones Agrarias, Recursos Naturales. 2:148-168.

Wareing, P. F. 1987. Phase change and vegetative propagation. pp. 263-270. <u>In</u> A. J. Abbott and R. K. Atkin (eds.) Improving Vegetatively Propagated Crops. Academic Press Limited, San Diego, CA.

White, P. R. 1967. Site of callus production in primary explants of spruce tissue. Am. J. Bot. 54:1055-1059.

Zimmerman, M. H. and C. L. Brown. 1971. Trees Structure and Function. Springer-Verlag, New York.

EFFICIENCY OF HORMONAL TREATMENTS FOR THE PROPAGATION
OF VIRGINIA PINE BY CUTTINGS

Q. Holifield [1], J. L. Ford-Logan [2], G. S. Foster [2], and G. F. Brown [1]

Abstract. This study was undertaken to evaluate the efficiency of growth regulator treatments to promote adventitious roots on cuttings of Virginia pine (Pinus virginiana Mill.) under favorable environmental conditions. Our long-term goal is to use conventional clonal propagation and micropropagation techniques to reduce the time required for producing greatly improved planting stock of Virginia pine for Christmas trees.

Cuttings of average diameter with a tip bud were randomly taken from current season's growth of superior second-generation Virginia pines and trimmed to a uniform length of 3 inches. The cuttings from 2-3 year old trees were inserted in a 2:1 (v/v) sphagnum peat moss and perlite rooting medium. Eight hormonal treatments were evaluated, which consisted of three IBA and/or DMSO combinations, two commercial rooting compounds, the fungicide Captan, Hare's Powder which is frequently used for rooting pines, and an untreated control. Data from two setting dates were used to verify results and to determine possible seasonal effects on rooting. The cuttings were set in a large open greenhouse rooting facility. The time of year in which the two rounds of cuttings were set allowed comparative determinations to be made on the better time of year for rooting Virginia pine cuttings. Each setting date consisted of eight treatments x four replications x six cutting plots giving a total of 192 treated cuttings per round.

After four months, cuttings were carefully removed from the media allowing a complete evaluation of the treatments and the developing root system. The results indicate that the summer months support poor root growth and development of cuttings of Virginia pine in Alabama and that Hare's powder appears to be one of the better hormonal treatments for rooting.

Keywords: Pinus virginiana, rooted cuttings.

INTRODUCTION

Virginia pine (Pinus virginiana Mill.) provides the southeastern United States with a renewable source of fiber, chemicals, and energy as well as aesthetic value to the landscape, watershed to farmlands, and natural habitats for wildlife. Its chief economic value is in timber and pulp paper production. The production of Virginia pine for Christmas trees has become a substantial industry throughout the southern states with approximately 300,000-400,000 seedlings being planting annually in the state of Alabama (pers. comm. G. F. Brown). Commercial growers are currently limited to using planting stock that was originally selected for its pulpwood characteristics. Growers desire planting improved genotypes of

---

[1] Graduate Research Assistant and Associate Professor, respectively, Department of Plant and Soil Science, Alabama A&M University, P. O. Box 1208, Normal, Alabama 35762.

[2] Plant Physiologist and Research Geneticist, respectively, USDA Forest Service, Southern Forest Experiment Station, Alabama A&M University, P. O. Box 1208, Normal, Alabama 35762.

higher quality stock material – more uniform trees that increase profitability. One strategy to improve the genetic qualities of Virginia pine for usage as Christmas trees is through the selection of genetically superior clones and their subsequent multiplication through vegetative propagation methods. Clonal propagation utilizes the total genetic variation that comprises a much larger effect (Foster and Shaw, 1987). In addition to the potential larger genetic gain, genetically improved plants could be delivered for large-scale planting several years before the availability of improved plants derived from seeds from the same tree source (Matheson and Lindgren, 1985). Since clonal trees will respond in a uniform manner under similar cultural treatments, this will aid the Christmas tree grower by simplifying crop maintenence and enhancing the quality of the trees produced.

The development of rooted cuttings from conifers is often a slow and difficult process. It has been demonstrated that hormone treatments are necessary for root initiation. The application of dimethyl sulfoxide (DMSO) has been used to promote a rooting reponse in several horticultural species (Edwards, 1979). DMSO increases the permeability of plant tissues to exogenously applied auxins (McKinniss, 1969). The hormone treatment that has given the better results in the rooting of pine species is the rooting powder developed by Hare (1971), of which the active ingredient is indolebutyric acid (IBA). To date, only one study (Snow and May, 1962) exits in the literature on the propagation of Virginia pine from cuttings, with time of year and hormone treatment affecting the degree of success in rooting.

The objective of this study was to evaluate the efficiency of various growth regulator treatments to promote the production of adventitious roots on stem cuttings of Virginia pine.

MATERIALS AND METHODS

Cuttings were randomly taken from second-generation select Virginia pine trees that were two-three years old from seed. The cuttings were three inches in length and of average diameter (3–4 mm) with an intact tip bud. The first group of cuttings was taken in March 1990 and the second group of cuttings was taken in June 1990. A randomized complete block design was used with eight treatments x four replications x six cuttings per plot giving a total of 192 treated cuttings per setting date. The treatments were as followed:

(1) Hare's powder[1]
(2) 4000 ppm IBA + 1% DMSO
(3) 4000 ppm IBA + 0% DMSO
(4)   0 ppm IBA + 1% DMSO
(5) 10% Captan powder
(6) 16.5% Dip 'N Grow[TM]
(7) Hormodin 3[TM]
(8) Untreated control

Cuttings were treated with the potassium salt of indolebutyric acid (K-IBA) with the IBA and DMSO treatments prepared using a 50% ethyl alcohol base. The Captan treatment was talc-based, similarly was Hormodin 3 containing 0.8% IBA. The Dip 'N Grow solution was mixed in distilled water.

The treated cuttings were set one inch deep in 10 inch^3 Leach Super Cells[TM] containing a 2:1 mixture of shredded sphagnum peat moss and perlite. They were subsequently placed in a temperature and humidity controlled greenhouse at International Forest Seed Company (Odenville, Alabama) with intermittent fogging and irrigation (Hughes, 1987).

---

[1] A talc formulation containing 1% each of the auxins IBA and 1-phenyl-3-methyl-5-pyrazolone (PPZ), 10% powdered sugar, 20% captan fungicide, and 1% of the growth retardant n-dimethylaminosuccinamic acid (B-Nine) (1-1-10-20-1).

After four months, percentage rooted cuttings per plot (RC) was assessed on a plot basis. A Chi-square (X^2) test using a 2 x 8 contingency table was conducted with data from each trial to assess treatment differences.

RESULTS AND DISCUSSION

A large difference in overall rooting was attributed to the seasonal and cultural environment effects. Differences among treatments were seen in the percent rooting of the March (Table 1) and June (Table 2) cuttings.

Table 1. Rooting percentages for March setting of Virginia pine cuttings.

| Treatment | Replication | | | | |
	1	2	3	4	x%
Hare's powder	16.7	66.7	50.0	50.0	45.8
4000 ppm IBA + 1% DMSO	0.0	0.0	0.0	16.7	4.2
4000 ppm IBA + 0% DMSO	40.8	0.0	0.0	0.0	10.2
0 ppm IBA + 1% DMSO	0.0	16.7	16.7	0.0	8.3
10% Captan	0.0	0.0	0.0	0.0	0.0
16.5% Dip 'N Grow™	0.0	16.7	16.7	33.3	16.7
Hormodin 3™	0.0	16.7	16.7	16.7	12.5
Untreated control	0.0	0.0	16.7	0.0	4.2

Overall x = 11.97%

Table 2. Rooting percentages for June setting of Virginia pine cuttings.

| Treatment | Replication | | | | |
	1	2	3	4	x%
Hare's powder	0.0	16.7	16.7	0.0	8.3
4000 ppm IBA + 1% DMSO	0.0	16.7	0.0	0.0	4.2
4000 ppm IBA + 0% DMSO	0.0	0.0	0.0	0.0	0.0
0 ppm IBA + 1% DMSO	0.0	0.0	0.0	16.7	4.2
10% Captan	0.0	0.0	0.0	0.0	0.0
16.5% Dip 'N Grow™	0.0	0.0	50.0	0.0	12.5
Hormodin 3™	0.0	16.7	0.0	16.7	8.3
Untreated control	0.0	0.0	0.0	0.0	0.0

Overall x = 3.8%

Although treatment differences were statistically non-significant (X^2 = 7.04 [NS]) for the March cuttings, the Hare's powder treatment proved to be the most effective with 45.8% of the cuttings rooted. The other treatments ranged from 0-16.7% rooting, which is more or less ineffective. The average rooting percentage for the March cuttings was 11.97% (Table 1).

The June cuttings gave a very poor result with non-significant treatment differences. The setting date for these cuttings was June 22, 1990. The average rooting percentage for the June cuttings was 3.8% (Table 2). The only conclusion that could be reached was, when using our system, Virginia pine cuttings should not be set for rooting during the hot summer months when cuttings are prone to dessicate and die.

As in many tree species, season of the year is an important factor in the rooting of cuttings. Snow and May (1962) achieved far greater rooting of IBA treated cuttings taken in December (72%) and March (18.6%) than we did for March and June cuttings. Their higher rooting percentage in March might be attributed to the use of past season's growth of much older material (eight or nine year old trees) versus the current season's growth from two-three year old trees in our study. Also, they may have set cuttings earlier in March, indicating that March may be too late to take cuttings from Virginia pine trees ready to flush, which may inhibit rooting.

Given our better results in March, we intend to repeat the study at various times during the November to March time period. DMSO with and without IBA needs to be further investigated along with other concentrations of IBA. The study by Mahalovich et al. (1987) showed that in the absence of auxin, DMSO treatments may increase permeability, possibly mobilizing endogenous auxins to produce high rooting percentages.

This study provides us with preliminary, yet very useful information on the rooting of Virginia pine. Requirements for optimum rooting need to be defined requiring further experimentation with the various parameters that effect rooting, pertaining to both donor and environment. Continuation of this effort will provide genetically improved Virginia pine trees to Christmas tree producers in the southeast.

ACKNOWLEDGEMENTS

LITERATURE CITED

Edwards, R. A. 1979. An evaluation of wounding and hormones on the rooting of cuttings. Royal N. Z. Inst. Ann. J. 7:74-82.

Foster, G. S. and D. V. Shaw. 1987. A tree improvement program to develop clones of lobloly pine for reforestation. Proc. 19th South. For. Tree Improv. Conf. pp. 17-21.

Hare, R. 1971. Factors promoting rooting of tree cuttings. Proc. 6th South. For. Phys. Workshop. Gainesville, FL. September 9-10, 1971.

Hughes, H. F. 1987. Cutting propagation of rust resistant hedges of Pinus taeda. Plant Prop. 1:4-6.

Mahalovich, M. F., S. E. McKeand and J. B. Jett. 1987. Seasonal rooting response of six-year-old loblolly pine clones to different concentrations of indol-3-butyric acid and dimethyl sulfoxide. Proc. 19th South. For. Tree Improv. Conf. pp. 125-131.

Matheson, A. C. and D. Lindgren. 1985. Gains from the clonal and the clonal seed-orchard options compared for tree breeding programs. Theor. Appl. Genet. 249-282.

McKinnis, G. R. 1969. The effects of DMSO as a carrier for IBA on the rooting of three juniper species. Plant Prop. 15:4-8.

Snow, Jr., A. G. and C. May. 1962. Rooting Virginia pine cuttings. J. For. 60:257-258.

CLONAL VARIATION IN ROOTING ABILITY OF VIRGINIA PINE

G.F. Brown[1], Q. Holifield[1], J.L. Ford-Logan[2], and G.S. Foster[2]

Abstract.--Twenty-four cuttings were taken from each of 25 clones of three-year-old Virginia pines (Pinus virginiana Mill.) previously selected for superior qualities as Christmas trees. The cuttings were arranged in a randomized complete block design with four replications and six cuttings per replication. All cuttings were treated with Hare's rooting powder and placed in a mist greenhouse at International Forest Seed Company (IFSCO) in Odenville, AL on March 6, 1990. After 5 months, all cuttings were moved to Alabama A&M University, where they were evaluated. Rooting percentages ranged from 0 to 79 percent, with highly significant differences among the 25 clones. The overall rooting percentage was 40 percent, with nine clones having better than 50% rooting.

To test the effect of a different cutting date, cuttings from 25 clones (10 clones repeated from the first experiment and 15 new clones) were taken on 3 August, 1990 and placed in the greenhouse at IFSCO. After 4 months, the cuttings were evaluated. Results of this experiment were then compared to the first experiment. The final objective is to select those clones that maximize both quality for Christmas tree production and rooting success. Selections will be used for the establishment of a new cutting orchard.

Additional Keywords: Pinus virginiana, vegetative propagation, cutting dates

INTRODUCTION

Virginia pine (Pinus virginiana Mill.) comprises a large portion of the Christmas tree production in the southeastern U.S., with annual sales totalling over 20 million dollars, yet genetic improvement of the species is progressing slowly compared with the emphasis on other southern pines of timber value. Clonal propagation, a commonly used horticultural technique, provides an avenue to relatively rapid genetic improvement of a species as well as resulting in a much more uniform crop, a highly

desirable feature in Christmas tree plantations. Production of genetically superior clones of Virginia pine emphasizing traits such as stem straightness, growth rate, and crown form, could represent a major advancement of the species for Christmas tree production.

Currently, cloning procedures which entail rooting of stem cuttings are the most cost effective methods available for forest tree species. An efficient rooting procedure is probably the most crucial factor in the entire production system. Genetic factors are known to control adventitious root formation in virtually all tested tree species (Foster 1990), and selection of superior clones must include rooting ability traits as well as growth traits (Foster et al. 1985). A single rooting trial with Virginia pine has been reported in the scientific literature, and it only assessed cultural factors governing adventitious root formation (Snow and May 1962). Knowledge is needed on which to base decisions regarding the strength of genetic control of rooting ability in Virginia pine in order to guide the design of clonal tree improvement programs.

The objectives of the current Virginia pine study were to:

(1) assess the degree of genetic control of rooting traits, and

(2) determine the reliability of clonal ranking for rooting ability.

MATERIALS AND METHODS

The clones tested in this study originated from within the Virginia pine tree improvement program being conducted at Alabama A&M University. Sample trees (ortets) were chosen at random from among second generation select trees in half-sib progeny tests of first generation select parents. No selection had been practiced for rooting ability. The ortets were located near Huntsville, Alabama.

Twenty-five trees were chosen on each of two dates during the second field season of the genetic test, hence the trees were 2 and 2-1/2 years old from seed, respectively. Trees selected at the second date included 10 of the same trees as in the first collection and 15 new trees. Rooting Trials 1 and 2 were initiated on March 6, 1990 and August 3, 1990, respectively.

The cuttings in both trials were rooted at International Forest Seed Co. (Odenville, Alabama) using their standard rooting procedure (Hughes 1987; Foster 1990). Stem cuttings were collected in the field, stored in ice chests, and transported to the greenhouse. Each cutting was standardized to a length of three inches, and each cutting had an intact tip bud. The basal 1 cm of the cutting was moistened with water and then dipped into Hare's (1974) talc-based rooting powder. Once treated, the cuttings were set 2-3 cm deep in 93 cm^3 plastic pots containing a 2:1 mixture of shredded peat and coarse perlite. Subsequently, the trays of cuttings were placed in a

greenhouse with intermittent fogging and irrigation (Hughes 1987). A randomized complete block design was used with clones as treatments and four replications of six cutting plots. All effects were considered to be random.

The cuttings were evaluated for rooting after four months in the greenhouse rooting environment. The number of rooted cuttings per plot was assessed on a plot basis.

Two separate analyses of variance were conducted. In the first, each rooting trial was analyzed separately using the form of analysis given in Table 1. The second analysis included only the ten clones common to both trials and used the form of analysis given in Table 2. Sums of squares were calculated using a least squares approach (PROC GLM, Type III, SAS Institute Inc. 1985) due to some imbalance in the data arising from unequal rooting among clones. Variance components were calculated by equating mean squares with expected mean squares (Kempthorne 1969). Coefficients of the variance components were adjusted for the data imbalance (Hartley 1967; Goodnight and Speed 1978).

Table 1. Form of the analysis of variance for the number of rooted cuttings of Virginia pine clones.

Source of variation	Degrees of freedom	Expected mean squares
Clones (C)	24	$\sigma^2_e + 4\,\sigma^2_C$
Reps (R)	3	$\sigma^2_e + 25\,\sigma^2_R$
Error	72	σ^2_e
Total	99	

Table 2. Form of the analysis of variance for the rooting of Virginia pine clones in a multiple trial study.

Source of variation	Degrees of freedom	Expected mean squares
Trials (T)	1	$\sigma^2_e + 4\,\sigma^2_{TC} + 40\,\sigma^2_T$
Clones (C)	9	$\sigma^2_e + 4\,\sigma^2_{TC} + 8\,\sigma^2_C$
T * C	9	$\sigma^2_e + 4\,\sigma^2_{TC}$
Reps (TC)	60	σ^2_e
Total	79	

Reliability of clone ranking for rooting traits was assessed in two ways. The magnitude and statistical significance level of the clone x trial interaction (Table 2) provided one assessment. Spearman's rank correlation (Sokal and Rohlf 1969) for clone means between trials provided the other evaluation.

Level of genetic control for each trait was determined by calculating broad-sense heritabilities. Broad-sense heritability was calculated for each trial separately on both an individual ramet basis (H^2) and also on a clone-mean basis ($H^2_{\bar{x}}$). Expected genetic gain was calculated as the product of broad-sense heritability on a clone mean basis and the selection differential.

$$H^2 = \frac{\sigma^2_c}{\sigma^2_c + \sigma^2_e} \qquad\qquad H^2_{\bar{x}} = \frac{\sigma^2_c}{\sigma^2_c + \frac{\sigma^2_e}{n}}$$

where,

σ^2_c = variance among clones

σ^2_e = error variance

n = number of ramets per clone

RESULTS AND DISCUSSION

The effect of clones on the rooting of Virginia pine cuttings was highly significant for both trials, and the estimated variance components were very similar (Table 3). In the second trial, the effect of replications was highly significant. The rooting success for the first replication was only 27 percent, compared to 50 to 53 percent success for the other three replications. No obvious reason for this difference could be deduced. Otherwise the results of both trials were very similar.

The general statistics for the experiment are presented in Table 4. The mean number of cuttings which rooted from groups of six is presented in the first column. It was assumed that a seven point (from 0 rooted to all six rooted) binomial distribution closely approximated a normal distribution. Therefore, this value was used for the analysis of variance. However, it is usually easier to comprehend and use average rooting percentages. Consequently, the rest of Table 4 (except the heritability estimates) is expressed in rooting percentages.

Table 3. Analysis of variance for the number of rooted cuttings from two trials of Virginia pine clones.

Source of variation	Degrees of freedom	Trial 1			Trial 2		
		Mean square	Variance component	% of total variance	Mean square	Variance component	% of total variance
Clones	24	5.36**	0.87	30.85	4.34**	0.78	31.84
Reps	3	3.65NS	0.07	2.48	14.70**	0.54	22.04
Error	72	1.88	1.88	66.67	1.13	1.13	46.12

** Significant at P<.01
NS Non-significant at P>.05

Table 4. Means[a], rooting percentages[b], ranges[b], heritabilities and select clone percentages[b] for two trials of Virginia pine clones.

Variable	Mean	Average Rooting			H2	H$\overline{x}^2$	Best 5 clones Mean
		Percentage	Max	Min			
Trial 1	2.56	42.67	75.00	12.50	0.32	0.65	69.17
Trial 2	2.75	45.83	87.50	16.67	0.41	0.73	70.83

[a]Mean per 6 cuttings
[b]Percentage of all 24 cuttings

Both trials had almost the same rooting percentage (43 and 46 percent, respectively). There was a large range in rooting percentage with relatively good rooting in some clones and poor in others. As stated above, the effect of clones was highly significant, and the relatively high heritability estimates indicate a strong genetic component. If the best five clones from either trial were used, then we should expect approximately 62 percent success on our rooting efforts. These results are very encouraging and lend support to our efforts to select the clones which will maximize rooting success. If select clones can be identified which will yield a 50 percent rooting success rate, then the establishment of cutting orchards may be a feasible way of producing improved plantlets.

The objective of this project was to determine the degree of genetic control of rooting and to determine the reliability of clonal ranks for rooting ability. Because of the need to screen as many clones as possible while providing some measure of repeatability, each trial only had 10 clones in common.

The relative ranks of rooted cuttings from the repeated 10 clones of Trial 2 were correlated with the results of Trial 1, resulting in a non-significant Spearman's rank correlation of -0.11. The results of Trial 2 were not correlated with Trial 1. In fact, if we use the results of Trial 1 to predict the outcome of Trial 2 under the assumption that the same clones should have the same rooting success rate, then a Chi-square test can be computed to compare the frequency of rooting success between the two trials. This test resulted in a Chi-square value of 35.14 at 9 degrees of freedom. There was a highly significant difference in the frequency of cuttings of the 10 clones between the two trials. This result is not encouraging. It was hoped that once a clone was selected based on superior rooting ability, that clone would maintain its superiority in future cutting trials.

The reason for the lack of repeatability is not known. It could be because the clones change relative rankings between trials. A clone that produces a high number of rooted cuttings in March may not do as well in August, whereas a clone with poor rooting success in March may do well in August. If this is true, a cutting orchard established based on one test may not yield good results if cuttings are taken at a different time of year or with different conditions. Another explanation for the lack of repeatability is that the 10 clones used for this part of the study are not representative of the entire population. We would then be observing random error.

If the first case is true, we would expect a fairly large clone by trial interaction term in our analysis of variance. However, from Table 5 it can be seen that this interaction was non-significant, along with the effects of the trial and the clones. The error term was large and accounted for 70 percent of the total variation. The clones are apparently not just shifting ranks. Because of this, the data from only the repeated clones were reanalyzed per trial in accordance with the form of the analysis of variance given in Table 1, except with only 10 clones instead of 25. In these analyses, the effect of the 10 clones was not significant in either trial. The probability of obtaining a larger F-test value was 0.11 in the first trial and 0.29 in the second trial. The randomly repeated 10 clones did not yield the same results as the larger group of 25 clones. With all 25 clones we obtained highly significant clonal effects which accounted for approximately 45.8% of the total variance (sums of squares clones divided by total sums of squares). The effect of the 10 clones was non-significant and accounted for approximately 25.4% of the total variance. The evidence indicates that the 10 clones used for this part of the study were not representative of the rest of the clones.

Table 5. Analysis of variance for the rooting of Virginia pine clones in a multiple trial study.

Source of variation	Degrees of freedom	Mean square	Variance component	% of total variance
Trial	1	6.61	6.61NS	3.68
Clone	9	25.56	2.84NS	14.22
T * C	9	22.26	2.47NS	12.39
Reps (TC)	60	125.25	2.09	69.70
Total	79			

This area obviously needs further study and more repeated trials. Another trial utilizing 30 clones with 20 repeat clones has already been set at IFSCO during the spring of 1991. Another trial is planned for later this year. The final goal is to screen for rooting ability at least 100 clones previously selected for Christmas tree characteristics and to obtain enough data to sufficiently judge the reliability of clonal ranking for rooting ability.

CONCLUSIONS

There appears to be a strong genetic component of the rooting success of juvenile Virginia pines. The overall rooting success of both trials was over 40 percent. Forty percent of all clones tested had rooting success of 50 percent or more. Selection of the top 5 clones would result in rooting success of approximately 50 percent. The selection of those clones with the highest rooting ability will greatly increase the efficiency of the clonal propagation process. This procedure appears to be a viable method of prcduction of genetically superior clones of Virginia pine for Christmas trees.

However, the reliability of these results from one trial to another is still in question. The rooting success of ten clones repeated over two cutting trials was not significantly correlated in these trials. Because of a non-significant clone by trial interaction term and a non-significant effect of clones in a re-analysis of only the ten repeated clones, it is believed that the ten clones may not be representative of all clones. This area should be further studied to obtain better conclusions.

LITERATURE CITED

Cochran, W.G. 1951. Testing a linear relation among variances. Biometrics 7:17-32.

Foster, G.S. 1990. Genetic control of rooting ability of stem cuttings from loblolly pine. Can. J. For. Res. 20:1361-1368.

Foster, G.S., R.K. Campbell, and W.T. Adams. 1985. Clonal selection prospects in western hemlock combining rooting traits with juvenile height growth. Can. J. For. Res. 15:488-493.

Goodnight, J.H., and F. M. Speed. 1978. Computing expected mean squares. SAS Institute Inc., Cary, NC. Tech. Rep. R-102.

Hare, R.C. 1974. Chemical and environmental treatments promote rooting of pine cuttings. Can. J. For. Res. 4:101-106.

Hartley, H.O. 1967. Expectation, variances and covariances of ANOVA mean squares by "synthesis". Biometrics 23:105-114.

Hughes, H.F. 1987. Cutting propagation of rust resistant hedges of Pinus taeda. Plant Propag. 1:4-6.

SAS Institute Inc. 1985. SAS user's guide: statistics, version 5. SAS Institute Inc., Cary, NC.

Snow, Jr., A.G., and C. May. 1962. Rooting of Virginia pine cuttings. J. For. 60:257-258.

Sokal, R. R., and F. J. Rohlf. 1969. Biometry. W. H. Freeman Co., San Francisco.

MATURATION OF DOUGLAS-FIR SOMATIC EMBRYOS
IN SUSPENSION CULTURES

R. Nagmani and R. J. Dinus[1]

Abstract.--Utility of different CHO sources and levels for
embryo maturation was evaluated with a Douglas-fir embryogenic
culture. The original explant was an immature embryo from an open-
pollinated seed orchard donor. Embryogenic tissues (250-300 mg
(fw)) were moved from agar-based maintenance medium through a
similar transition medium, containing 1% activated charcoal and
lacking growth regulators, to 50 ml of liquid maturation media in
250 ml Erlenmeyer flasks. The maturation medium, a modified MS
medium, contained half-strength micro- and macro-nutrients,
included L-glutamine in lieu of ammonium nitrate, lacked growth
regulators, and was supplemented with four sucrose, maltose, and
glucose levels. One sucrose and one maltose level were tested with
and without ABA. Cultures were incubated on a platform shaker (120
rpm); 16 hr photoperiod (soft-white fluorescent), 26 +/- 2°C.
Cultures were transferred to fresh media, and embryo status was
observed weekly.

Of the three CHO sources, only maltose advanced early-stage
embryos present on maintenance and transition media to
cotyledonary and/or mature stages. Glucose did not promote
development to any significant extent. Sucrose had marginal
effects with only one level, higher than routinely used in plant
cell culture, moving small embryo numbers to advanced but still
precotyledonary stages. All maltose concentrations moved numerous
embryos to these stages within four weeks and, in turn, to
maturity by the end of the six-week culture period. Embryo
numbers, as well as morphologies, varied among maltose levels,
with the lowest level (0.08M) maturing the largest number (82 per
50 ml of medium). Culture in liquid media also reduced the
tendency of embryos to remain fused to one another as often occurs
on agar-based media.

Keywords: Pseudotsuga menziesii (Mirb.) Franco, embryogenesis,
plant cell and tissue culture, suspension cultures, abscisic acid,
carbohydrates, maltose.

Abbreviations: CHO = Carbohydrates, ABA = Abscisic Acid, MS =
Murashige and Skoog, 2,4-D = 2,4-Dichlorophenoxyacetic acid, BA =
6-Benzylaminopurine.

---

[1] Assistant Professor and Professor, respectively. Institute of Paper
Science and Technology, 575 14th Street, NW, Atlanta, GA 30318.

INTRODUCTION

Somatic embryogenesis has been obtained in a variety of conifers since first reported for Norway spruce (_Picea abies_ (L.) Karst) (Hakman et al. 1985) and European larch (_Larix decidua_ Mill.) (Nagmani and Bonga 1985). Repeatable protocols, often with provisions for quantification, have been published for several species, e.g., Norway spruce (Becwar et al. 1987, 1988), white spruce (_Picea glauca_ (Moench) Voss) (Hakman and Fowke 1987, Webb et al. 1989), white pine (_Pinus strobus_ L.) (Finer et al. 1989), and coastal redwood (_Sequoia sempervirens_ (D. Don) Endl.) (Bourgkard and Favre 1988).

Success with Douglas-fir and loblolly pine (_Pinus taeda_ L.), however, has been limited, particularly with those steps involving embryo development and maturation. In earlier work with Douglas-fir, we obtained a few embryos, but many were fused together or otherwise abnormal (Nagmani et al. In press). A recent patent (Gupta and Pullman 1990) indicates that osmolarity of culture media plays a critical role in the maturation step. Addition of sugars, hexitols, or cyclitols capable of sufficiently raising osmotic potential greatly improved maturation of embryos of Norway spruce, Douglas-fir, and loblolly pine. In Douglas-fir, mature embryos and plantlets were obtained from a variety of genotypes. CHO source and ABA have also been tested in our laboratory. Substitution of maltose or glucose for sucrose in maturation media yielded fully developed loblolly pine embryos. Small but significant numbers were recovered, and all had normal phenotypes, morphologically and anatomically (Uddin et al. 1990).

These several findings prompted further investigation of CHO effects on somatic embryo maturation. This report describes results from preliminary experiments designed to extend our best loblolly treatments to Douglas-fir and to evaluate them in a suspension culture system.

MATERIALS AND METHODS

The embryogenic culture used in the present trial was initiated from an immature embryo in summer 1988 (Nagmani et al. In press). Developing open-pollinated cones from the seed orchard donor tree, WTC-570, were supplied by Weyerhaeuser Company. Initiation occurred on MSCG media (Table 1) supplemented with 5.0 mg/L 2,4-D and 2.5 mg/L BA.

Maintenance and proliferation were executed per Nagmani et al. (In press), with the following exceptions. Vigorous growth, especially after the first 1.5 years, required trial and error adjustment of the MSCG medium. Consistent performance was obtained by halving micro- and macro-nutrient concentrations and reducing the level of 2,4-D to 2 mg/L. The complete formulation (Table 1) is hereafter referred to as mMSCG.

Table 1. Basal media, supplements, and growth regulator combinations for initiation, maintenance, and maturation of Douglas-fir somatic embryos. Composition of MS media is shown for comparison.

Components, mg·L^{-1}	MS	MSCG5/2.5	mMSCG2/0	mMSG[1]
NH_4NO_3	1650	--	--	--
KNO_3	1900	100	950	950
$MgSO_4 \cdot 7H_2O$	370	370	185	185
KH_2PO_4	170	170	85	85
$CaCl_2 \cdot 2H_2O$	440	440	220	220
$Ca(NO_3)_2 \cdot 4H_2O$	--	--	--	--
KCl	--	745	--	--
KI	0.83	0.83	0.41	0.41
H_3BO_3	6.2	6.2	3.1	3.1
$MnSO_4 \cdot H_2O$	22.3	22.3	11.15	11.15
$ZnSO_4 \cdot 7H_2O$	8.6	8.6	4.3	4.3
$Na_2MoO_4 \cdot 2H_2O$	0.25	0.25	0.125	0.125
$CuSO_4 \cdot 5H_2O$	0.025	0.025	0.0125	0.0125
$CoCl_2 \cdot 6H_2O$	0.025	0.025	0.0125	0.0125
$NiCl_2 \cdot 6H_2O$	--	--	--	--
$FeSO_4 \cdot 7H_2O$	27.8	27.8	27.8	27.8
Na_2EDTA	37.3	37.3	37.3	37.3
Inositol	100	100	100	100
Glycine	--	--	--	--
Nicotinic acid	0.5	0.5	0.5	0.5
Pyridoxine	0.1	0.1	0.1	0.1
Thiamine HCl	0.1	0.1	0.1	0.1
Sucrose	30,000	30,000	30,000	--
Glutamine (G)	--	500	500	500
Casein Hydrolysate (C)	--	1000	1000	--
Agar	0.8%	0.8%	0.8%	--
Growth Regulators				
2,4-D	--	5	2	--
BA	--	2.5	--	--
Kinetin	--	--	--	--
ABA	--	--	--	2.6

[1] See text for CHO sources and concentrations used in mMSG maturation medium.

Maturation was accomplished in two steps: a transition step to remove growth regulators used for maintenance and proliferation, and the actual maturation step in which various CHO and ABA treatments were tested. The first, or transition, step involved incubation in the dark for seven days on mMSCG supplemented with 1 percent activated charcoal and gelled with 0.8

percent agar. All cultures were treated the same during the maintenance and transition steps to ensure consistent composition before transfer to experimental maturation media.

The second, or maturation, step involved incubation on modified mMSCG medium. Modifications consisted of not adding casein hydrolysate and using a liquid, rather than gelled, form. The formulation (Table 1) is hereafter referred to as mMSG. To evaluate CHO effects, mMSG was supplemented with four different levels each of sucrose (control) and maltose (0.08, 0.17, 0.31, and 0.49 M) and glucose (0.16, 0.33, 0.49, and 0.66 M).

To glimpse ABA effects, 0.08 M sucrose and maltose treatments were tested with and without ABA (10 μM). This level of sucrose (3 percent w/v) is used widely in plant tissue culture systems, can be regarded as a control or standard, and was therefore considered a logical starting point for testing growth regulator supplements.

Weighed masses of embryogenic tissues (250 to 300 mg (fw)) were transferred from maintenance medium first to transition medium, and then dispersed in 50 ml of mMSG maturation medium in 250 ml Erlenmeyer flasks. Cultures were incubated on a platform shaker (120 rpm) in a lighted culture room; 16 hr photoperiod (soft-white fluorescent) and 26 +/- 2 °C. Sub-culturing was done every seven days.

Individual treatments were represented by four flasks (replications). One flask from each treatment, chosen at random, was used to monitor development. A 3 ml aliquot was withdrawn from each such flask every seven days for microscopic observation. Embryo stages and numbers per stage were recorded. Stage identification followed the convention of Buchholz and Stiemert (1945). Observations from all flasks were taken at the end of the overall experiment, except for the few lost to contamination. The experiment was terminated at six weeks as treatments yielding responses had produced mature somatic embryos by that time or earlier.

RESULTS AND DISCUSSION

Embryos present in tissues on maintenance medium were at Stage 1, the very earliest stage of development (Figure 1A). Incubation on maintenance and transition media solidified with agar generally resulted in fusion of two or more embryos (Figure 1B). In earlier work (Nagmani et al. In press), this same phenomenon was observed whenever solid media were used. Even after transfer to liquid maturation medium (mMSG) in the present research, embryogenic tissues were slow to disassociate. By the first formal observation date, seven days after transfer to this medium, however, microscopic examination showed numerous embryos clearly free of one another (Figure 1D and E).
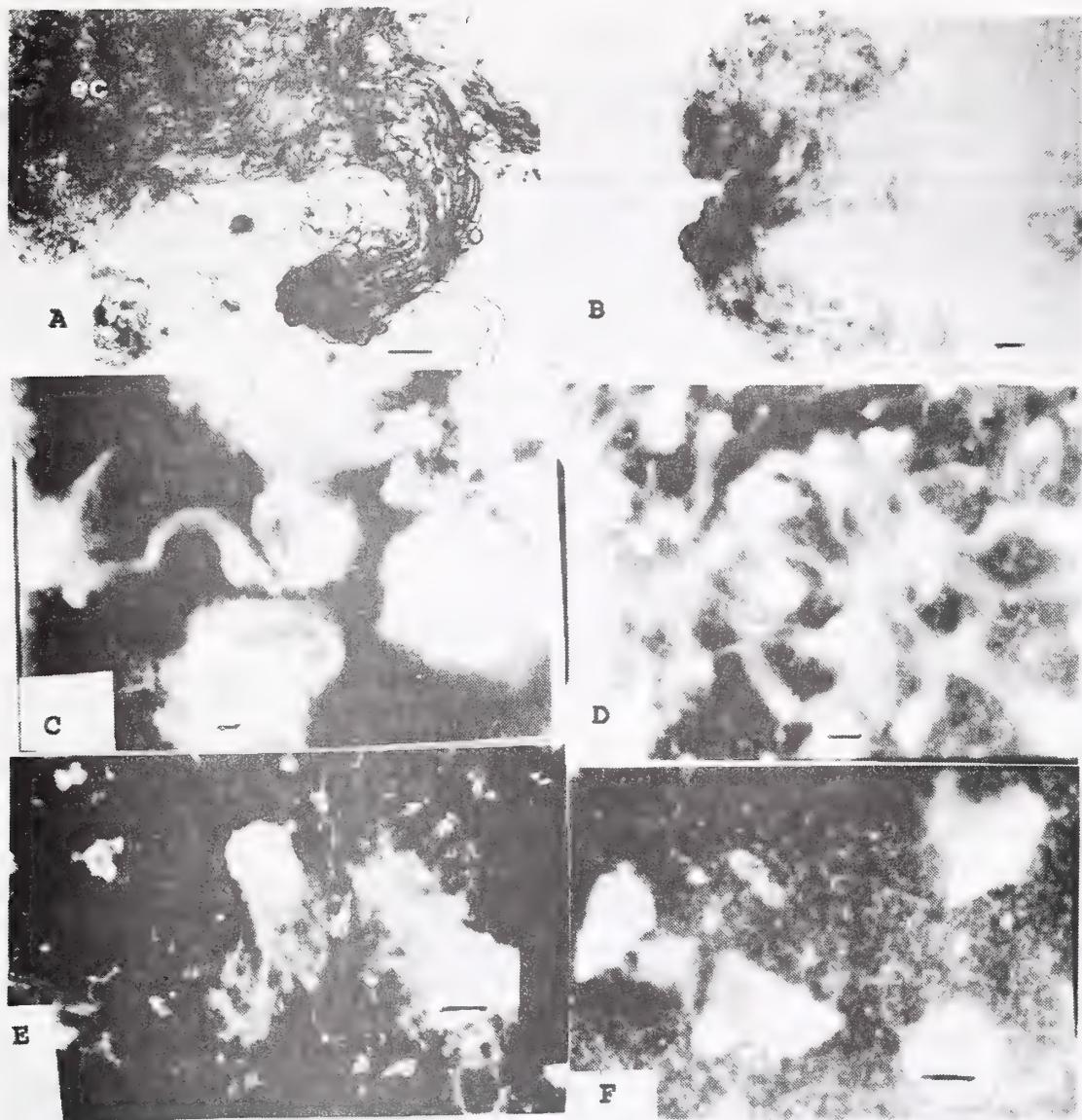
Figure 1. Maturation of Douglas-fir somatic embryos.

A.   Early stage embryo (Stage 1) on agar-based maintenance medium. Scale
      bar = 100 µm.
B.   Small group of embryos adhering together on agar-based transition
      medium. Scale bar = 50 µm.
C.   Stage 2 and 3 embryos after 4 weeks in liquid maturation medium
      containing 0.31 M sucrose. Scale bar = 1 mm.
D.,E. Stage 2 and 3 embryos after 4 weeks in liquid maturation medium
      containing 0.08 M maltose and 10 µM ABA.
F.   Mature cotyledonary embryos (Stages 5-7) after 6 weeks in maturation
      medium supplemented with maltose but without ABA. Scale bar = 1 mm.

Of the three CHO sources, only maltose advanced embryos to the cotyledonary and mature stages (Table 2). Glucose did not promote development to any significant extent. As in past trials (Nagmani et al. In press), sucrose effects were marginal. Incubation with 0.31 M sucrose moved a small number of embryos to, but not beyond, Stage 3, a still precotyledonary stage (Figure 1C). These may have developed further, but this and several related treatments became contaminated and were terminated at the end of the fourth week. In view of these and our earlier findings with sucrose, higher than standard concentrations of sucrose and/or glucose warrant reexamination.

Table 2. Maturation of Douglas-fir somatic embryos after 4 and 6 weeks in suspension cultures supplemented with various CHO concentrations with and without ABA.[1]

Treatment Combination		Weeks in Culture:	4	6
Maltose	+/- ABA	Embryo Stage:	2-3	5-7
(M)	(10 µM)		--- Mean No. per 50 ml ---	
0.08	+		151	[2]
0.08	-		190	82
0.17	-		145	55
0.31	-		155	60
0.49	-		185	49

[1] Sucrose and glucose supplements moved only a very few embryos to stages beyond those observed on maintenance medium.

[2] Treatment terminated at four weeks as a result of contamination.

In contrast to sucrose, and regardless of concentration, maltose moved numerous embryos to Stages 2 and 3 by end of the fourth week (Table 2). Many of these, in turn, were advanced to Stage 5 and/or beyond by the sixth week. Relative numbers and morphology varied among maltose concentrations. As an example, the lowest concentration (0.08 M) produced the largest numbers, but many were green and had elongating radicles (Figure 1F) - a potential symptom of precocious germination.

Addition of 10 µM ABA along with 0.08 M maltose produced similar effects by the end of the fourth week. Fate of embryos exposed to ABA, regrettably, could not be observed beyond that point, as contamination forced early termination of the treatment. We speculate that they would have developed further and that precocious germination would not have occurred.

Findings from these preliminary experiments with Douglas-fir suggest, at least for this one genotype, that maltose fosters somatic embryo maturation, that ABA may also play an important role, and that suspension cultures offer significant advantages over solid media. Results concerning maltose and ABA agree with our earlier work on loblolly pine (Uddin et al. 1990), where maltose (or glucose) in combination with ABA was superior to sucrose. Whether

these alternative sugars are better energy sources than sucrose, regulate media osmolarity, or act by some other mechanism remains uncertain. According to Gupta and Pullman (1990), timed addition of selected CHO additives adjusts osmotic potential of culture media, thereby governing course and pace of development. Future research, involving an array of genotypes, should seek to confirm the best CHO additives, better define needed concentrations, clarify advantages of liquid over solid media, and determine mechanisms underlying such modifications.

ACKNOWLEDGMENT

LITERATURE CITED

Becwar,M.R., T.L.Noland, and S.R.Wann. 1987. A method for quantification of the level of somatic embryogenesis among Norway spruce callus lines. Plant Cell Reports. 6:35-38.

Becwar,M.R., S.R.Wann, M.A.Johnson, S.A.Verhagen, R.P.Feirer, and R.Nagmani. 1988. Development and characterization of in vitro embryogenic systems in conifers. P. 1-18 in Somatic cell genetics of woody plants, M.R.Ahuja (ed.). Kluwer Academic Publishers, Dordrecht, The Netherlands.

Bourgkard,F. and J.M.Favre. 1988. Somatic embryos from callus of Sequoia sempervirens. Plant Cell Reports. 7:445-448.

Buchholz,J.T. and M.L.Stiemert. 1945. Development of seeds and embryos in Pinus ponderosa with special reference to seed size. Illinois Academy of Science Transactions. 38:27-50.

Finer,J. J., H.B.Kriebel, and M.R.Becwar. 1989. Initiation of embryogenic callus and suspension cultures of eastern white pine (Pinus strobus L.). Plant Cell Reports. 8:203-206.

Gupta,P.K. and G.S.Pullman. 1990. Method for reproducing coniferous plants by somatic embryogenesis. U.S. Patent No. 4,957,866. 14 p.

Hakman,I., L.C.Fowke, S.von Arnold, and T.Eriksson. 1985. The development of somatic embryos in tissue cultures initiated from immature embryos of Picea abies (Norway spruce). Plant Science. 38:53-59.

Hakman,I. and L.C.Fowke. 1987. Somatic embryogenesis in Picea glauca (white spruce) and P. mariana (black spruce). Can. J. Bot. 65:656-659.

Nagmani,R. and J.M.Bonga. 1985. Embryogenesis in subcultured callus of Larix decidua L. Can. J. For. Res. 15:1088-1091.

Nagmani,R., M.A.Johnson, and R.J.Dinus. In press. Effect of explant and media on initiation, maintenance, and maturation of somatic embryos in Pseudotsuga menziesii (Mirb.) Franco (Douglas-fir). In Woody plant biotechnology, M.R.Ahuja (ed.) (Proc. IUFRO Somatic Cell Genetics Working Party and NATO Advanced Research Workshop on Woody Plant Biotechnology, Placerville, CA, Oct. 15-19, 1989.). Plenum Publishing Corp., New York.

Uddin,M.R., R.J.Dinus, and D.T.Webb. 1990. Loblolly pine somatic embryo development and maturation. Abstract in IUFRO World Congress Proc., Montreal, Canada.

Webb,D.T., F.Webster, B.S.Flinn, D.R.Roberts, and D.E.Ellis. 1989. Factors influencing the induction of embryogenic and caulogenic callus from embryos of Picea glauca and P. engelmanii. Can. J. For. Res. 19:1303-1308.

GENERAL SESSION IV

GENETIC TESTING AND SELECTION

MORPHOGENETIC SUBDIVISION OF HEIGHT GROWTH AND EARLY SELECTION IN MARITIME PINE.

A. Kremer, M. Lascoux, A. Nguyen
INRA, Laboratoire de génétique et d'amélioration des arbres forestiers, Pierroton, 33610-Cestas, France.

Abstract As part of ongoing studies on early selection in maritime pine, we have summarized the major points so far analyzed namely (i) optimal age for selection with traditional technics (ii) identification of potential early selection criteria (iii) choice of juvenile testing environments. When total height is used as criteria, experimental results and simulations show that selection should be done later than 12 years. Subdivision of height into components shows that gene action may vary with components and that there is a high degree of genetic plasticity in height growth. As a result identification of a single component as selection criteria is doubtful. Explicative approaches show that raising first season seedlings in continuous days accelerates the maturation of the seedlings. This treatment exhibits genetic differences among different families that are associated with adult performances.

Keywords: *Pinus pinaster* Ait., height growth, early selection, morphogenetic components

INTRODUCTION

The maritime pine breeding program was started in the late fifties by the pioneer work of G.Illy (1966). Continued by Baradat (Baradat and Pastuzka, 1990), it has nowadays reached the third generation. The experience has shown that the generation duration is varying between 12 and 15 years. As data have accumulated on the older progeny tests, it has become clear that early evaluation is among the greatest challenge of the actual tree improvement program.

The present review attempts to summarize the research conducted in the field of early selection for height growth. Facts and results will be presented refering to the three major issues associated to early selection: (i) optimal age for selection based on traditional technics, (ii) identification of potential early selection criteria, (iii) choice of juvenile environmental conditions for testing.

The general strategy followed in this research program is the subdivision of height growth in different additive and multiplicative components. Besides the perspective of early selection, this approach has the advantage to afford basic information on the height growth process *per se*. The lack of this information is still a major drawback which has limited progress in physiological genetics of height growth in forest trees. Concerning early selection strictly, two specific

203

features of the subdivision of height growth have been stressed: the cumulative and sequential nature of growth on the one hand and the genetic architecture of height growth on the other hand.

1. JUVENILE-MATURE CORRELATION: A CHALLENGE FOR MARITIME PINE BREEDING.

The data presented here report on the important effects of juvenile-mature correlation on tree improvement efficiency. They are based on age-age correlation estimated in one of the oldest open pollinated progeny test established in the Landes area. The progeny test comprises 100 open pollinated of trees selected in natural stands of maritime pine during the late fifties. These parental trees originated from various natural stands and were older than 40 years and close to rotation age. Among the 100 families, 88 belong to 44 pairs of families choosen as follows (Illy, 1966):

* one member of a pair corresponds to the offspring of plus trees, selected on the basis of its relative superiority over the 30 nearest trees in the stand.

* the second member corresponds to the offspring harvested on the control tree among the nearest neighbours: the control tree has its total height closest to the overall mean of the neighbours.

Data available on the parental trees were the standardized value of total height (relative to the population of the 30 nearest neighbours). Successive total heights between age 2 and age 22 were measured on the offsprings in the progeny test. Figure 1 shows the evolution of the proportion of pairs in which the offspring of the plus tree were superior to the offspring of the control tree. At age 2 of the progeny test, this proportion was only 50%, which would be expected if there was no correlation between age 2 and the adult stage. This proportion constantly increased with age of the progeny test reaching 70% at age 22. Correlation coefficients between standardized values of total height of the parental trees and mean values of their offspring provide a similar picture of JM correlation (figure 2). These correlation coefficients can be considered as the best estimates actually available on genetic JM correlation. They include GE interactions, since the parental trees came from different forests. There is a definitive trend of increase with the age of the progeny test, starting from -.1 at age 2 and reaching .33 at age 22.

From these results, one may conclude that (i) phenotypic selection of adult trees for height growth was effective and that (ii) selection earlier than 12 years based on total heights may lead to genetic gain close to zero.
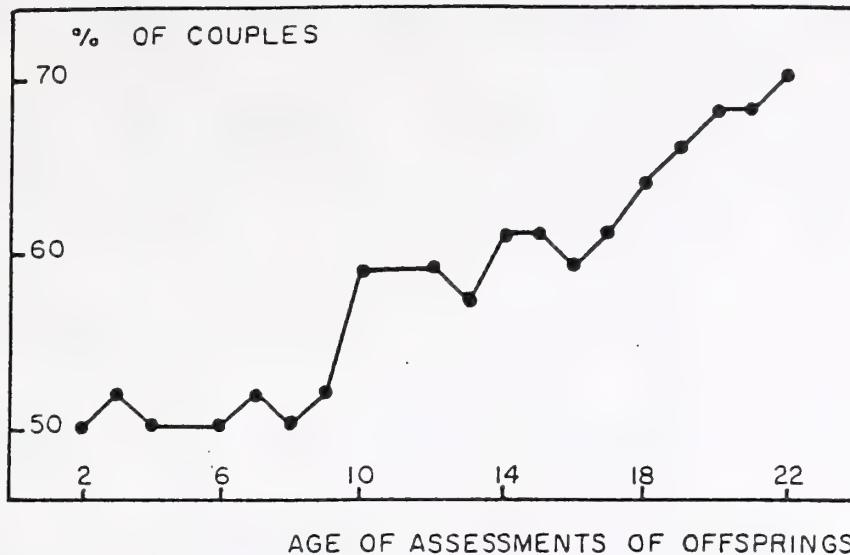
Figure 1. Percentage of pairs (plus and control tree) for which the offsprings of the plus trees are higher than the offsprings of the control tree. The progenies are measured every year from the second to the 22nd growing season.
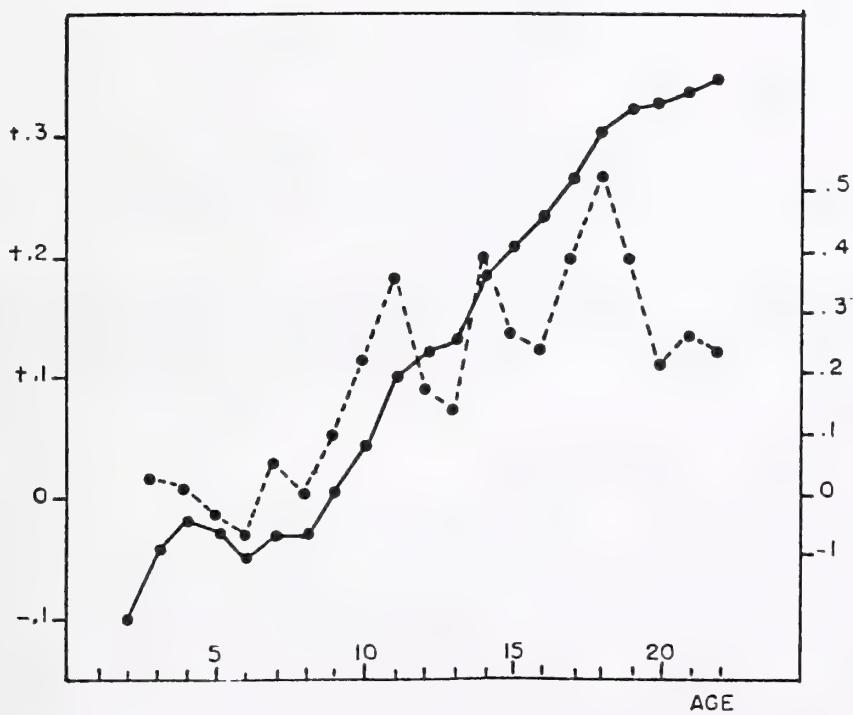
Figure 2. Evolution of the correlation coefficient between the parental standardized values at harvest age and the mean of total height (———) or AHI (— — — —) of the open pollinated families. Scale of correlation with TH is on the left y axis of the diagram, on the right y axis for AHI.

2. A STRATEGY FOR EARLY EVALUATION: SUBDIVISION OF TOTAL HEIGHT INTO MORPHOLOGICAL AND MORPHOGENETICAL COMPONENTS.

Total height (TH) of a tree is a cumulative trait that can be subdivided in various components from the total height up to the cellular level (Cannell, 1978; Kremer and Lascoux, 1988).

(1) First level of subdivision:

$$TH = \Sigma \; AHI$$

AHI : Annual height increment

(2) Second level of subdivision

$$AHI = PSL + AESS*SSL$$

PSL : Primary shoot length, corresponding to the first morphogenetic cycle (for definition of a cycle see Debazac, 1963)
AESS: ability to express additionnal cycle (yes or no response)
SSL : length of shoots corresponding to additional cycles.

(3) Third level of subdivision:

PSL= NSU*MSUL
SSL= NSU*MSUL

NSU : Number of stem units (see Doak, 1935 for definition)
MSUL: Mean stem unit length

The subdivision of height growth into components can lead to two different approaches for designing a strategy for early evaluation. The first is related to the cumulative nature of height growth, the second to the genetic architecture of height growth.

Cumulative nature of height growth.

There is a mechanistic relationship between components and the composite traits. This feature can be used in the definition of a strategy for identifying the optimal age for selection. For example, since components at a given age are part of total height at rotation age, how many components over how many years should be summed to provide an adequate prediction of total height at rotation age? We report here on the results obtained with this strategy applied at the first level of subdivision.

Genetic architecture of height growth.

By subdividing total height into components, one may ask if some components benefit of genetic parameters at a high level: heritability, contribution and genetic correlation with the composite trait. This approach may lead to identify potential early selection criteria. The estimation of these genetic parameters in the frame of the subdivision is called "genetic architecture of height growth".

3. CUMULATIVE NATURE OF HEIGHT GROWTH AND OPTIMAL AGE FOR SELECTION

3.1. A model of age-age correlations based on serial correlations of annual height increments.

Since height growth is a cumulative trait, it is obvious that the genetic correlation between two different total heights at age t and t' depends strictly on the correlation between the different annual height increments comprised in the total heights. The model that will be developed is based on the correlation between annual height increments. For simplicity of the formulas total height at age t (TH_t) is now written as Y_t and AHI_t as X_t. Three different informations are needed to construct the model.

(1) The serial genetic correlation structure, i.e. the variation of r (X_t , $X_{t'}$) as a function of age (t) and of lag between both increments (t-t'). r (X_t , $X_{t'}$) is the genetic (additive) correlation between annual height increments at age t and t'.

(2) The variation of the genetic coefficient of variation of the annual height increments ($CV(X_t)$) with age t, i.e. the variation of genetic control of AHI with age.

$$CV(X_t) = \frac{\sqrt{V_{At}}}{\overline{X}_t}$$

V_{At} is the genetic (additive) variance of the annual height increment at age t.

$\overline{X}_t$ is the phenotypic mean value of the annual height increment at age t.

(3) A phenotypic growth model of total height providing the evolution of X_t as a function of t.

The covariance between genetic values of annual height increment t and t' can then be written as :

$$Cov(X_t , X_{t'}) = \overline{X}_t \ \overline{X}_{t'} \ CV(X_t) \ CV(X_{t'}) \ r(X_t , X_{t'})$$
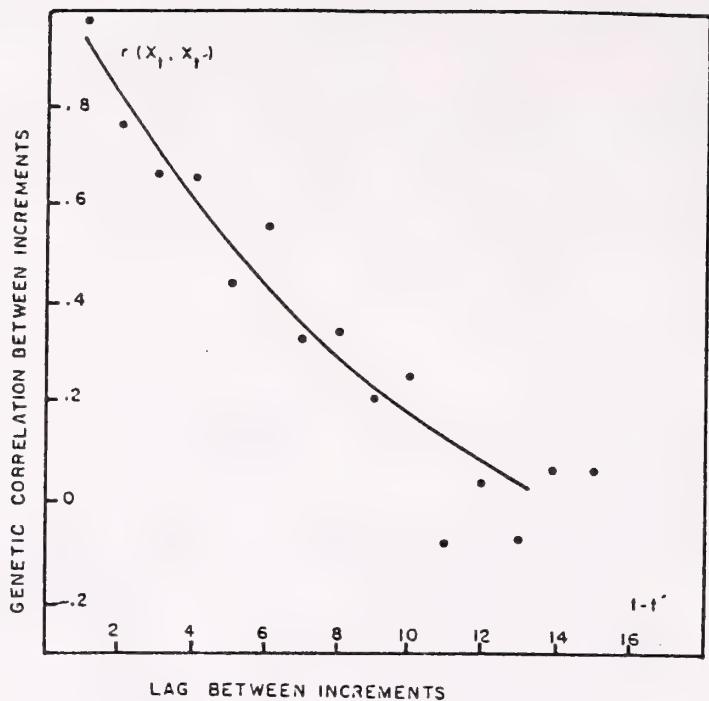
Figure 3a. Genetic correlation values between successive AHIs
as a function of the lag separating them (from lag 1 to lag
15). Dots represent observed points. The data come from the
progeny test outlined in paragraph 1. For lag 1, the dot
represents the mean of 20 values of coefficient of correlation
between AHIs separated by only one year. For lag 15 a mean of
only 6 values are available. The line drawn is fitted with
polynomial function. This serial correlation structure is
refered on figure 4a and 4b as case1.
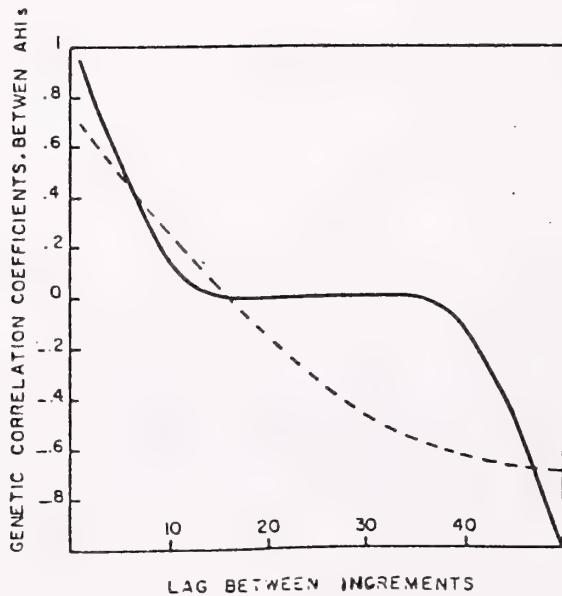
Figure 3b. Two examples of theoretical serial correlation
structure tested in the age-age correlation model. (————— :
case2; ~~~~~: case3)
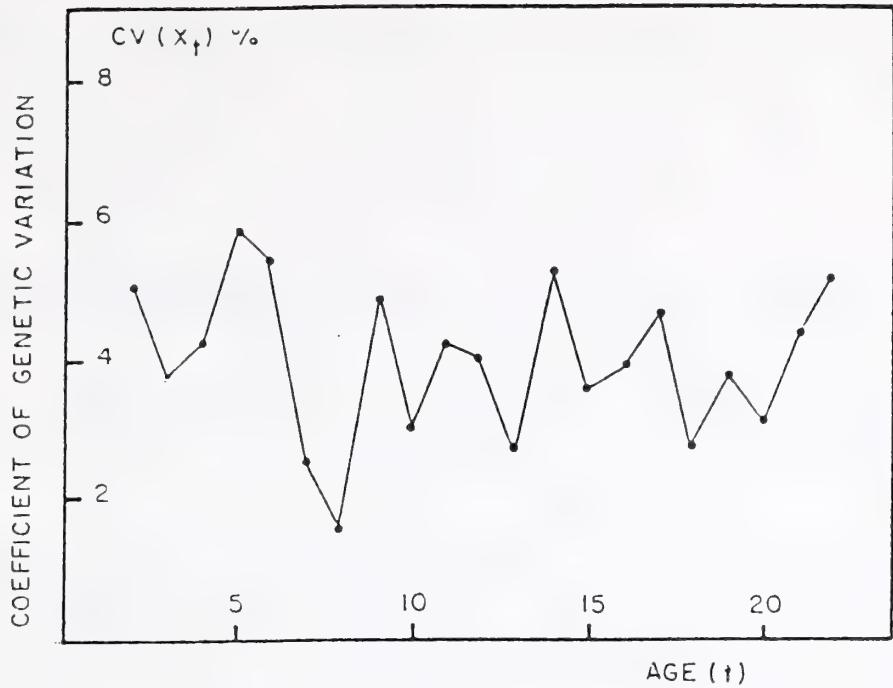
Figure 3c. Evolution of the genetic (additive) coefficient of variation of successive AHIs. Data come from the progeny test described in paragraph 1.
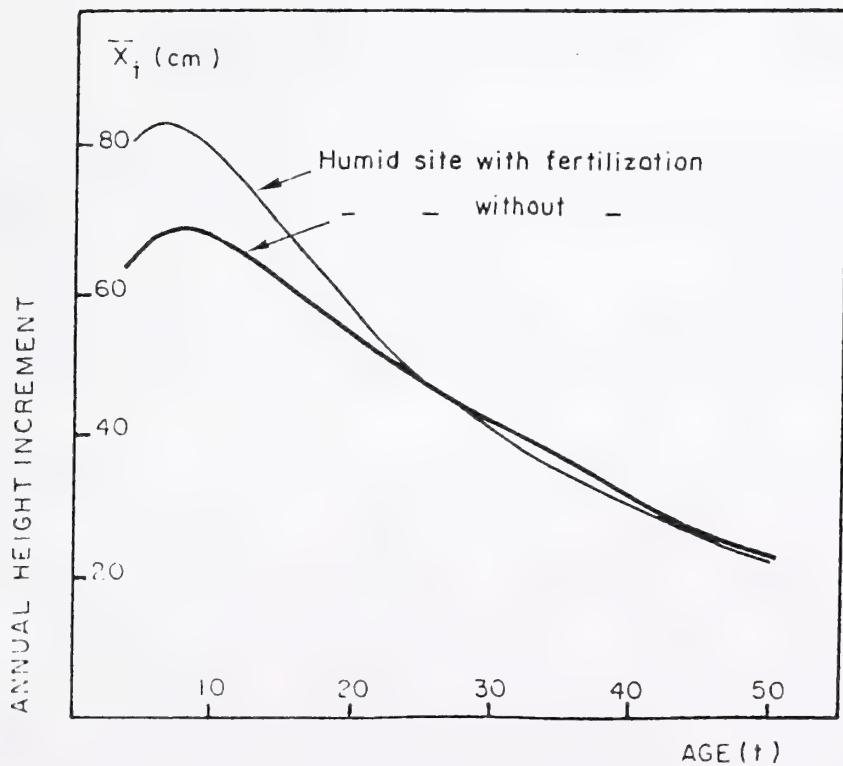
Figure 3d. Phenotypic height growth model in two different forest situations. Data are kindly provided by B. Lemoine.

As a result the covariance between total heights at different ages t and t' (t'<t) is :

$$\text{Cov}(Y_t , Y_{t'}) = \sum_{i=1}^{t'} V(X_i) + 2 \sum_{i=1}^{t'} \sum_{j=1}^{t'} \text{Cov}(X_i , X_j)$$

$$+ \sum_{j=1}^{t'} \sum_{i=1}^{t} \text{Cov}(X_i , X_j)$$

The matrix of genetic correlation between any couple of total heights can now be calculated from the matrix of covariances of annual height increments.

3.2. Application of the model in various situations.

Various situations are tested according to different values of the three inputs in the model.

(1) Serial genetic correlation structure. We assumed that genetic correlations between different ages depend only on the lag separating them (t-t') and not on age (t). Observed serial correlations are shown on figure 3a. Data originate from the progeny test described in paragraph 1 . Genetic correlations between AHIs constantly decrease reaching 0 when then are separated by more than 13 years. Three situations are tested:

- serial correlations as observed in figure 3a. We further assume that when lag is superior to 13 years the correlations remain null.
- the second and third situations are theoretical situations shown in figure 3b. Compared to situation 1, serial correlations can become negative .

(2) Genetic coefficient of variation. We assume that their values do not change with age, as suggested by observed values shown on figure 3c: between age 2 and 22 no definite age pattern can be observed. The value of .04, corresponding to the average values in figure 3c is used in the model.

(3) Data available from sylviculturists (Lemoine, 1981) are used to construct a phenotypic growth model correponding to two contrasting situations : humid sandy moor with and without fertilization (figure 3d).

Curves of figure 4, resulting from calculations outlined in paragraph 2.1 show that age-age correlation based on the model of serial correlation are rather optimistic. Interestingly they follow the pattern of Lambeth's empirical law (Lambeth, 1980). Even in the case when AHIs are negatively correlated over large lags (situation 3 of serial correlation structure), the correlation of total height between age 10 and 50 varies between .20 and .6. However these values are higher than those represented in figure 2. Several reasons may explain the discrepancy: simplification of the assumptions in the model of age-age correlation, GE interaction...From figures 2 and 4, one may conclude that selection based on total height should be achieved when trees are older than 12 years.

210
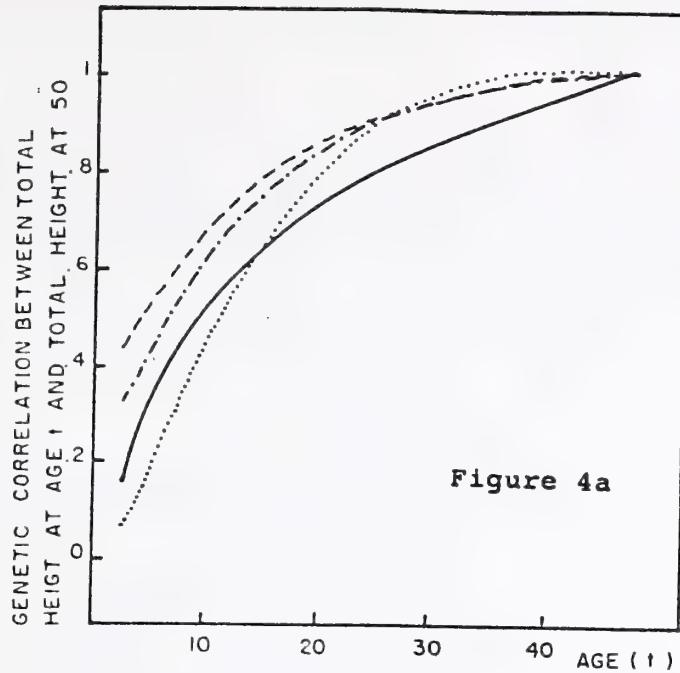
Figure 4 Simulated age-age genetic correlation between total
height at age t and height at rotation age (age 50).
Calculations are made for humid sandy moor with (figure 4a) and
without (figure 4b) fertilization. CV is held constant at .04.
  – – – – : serial correlation structure case1
–·–·–··: serial correlation structure case2
·········: serial correlation structure case3
————— : Lambeth's empirical law
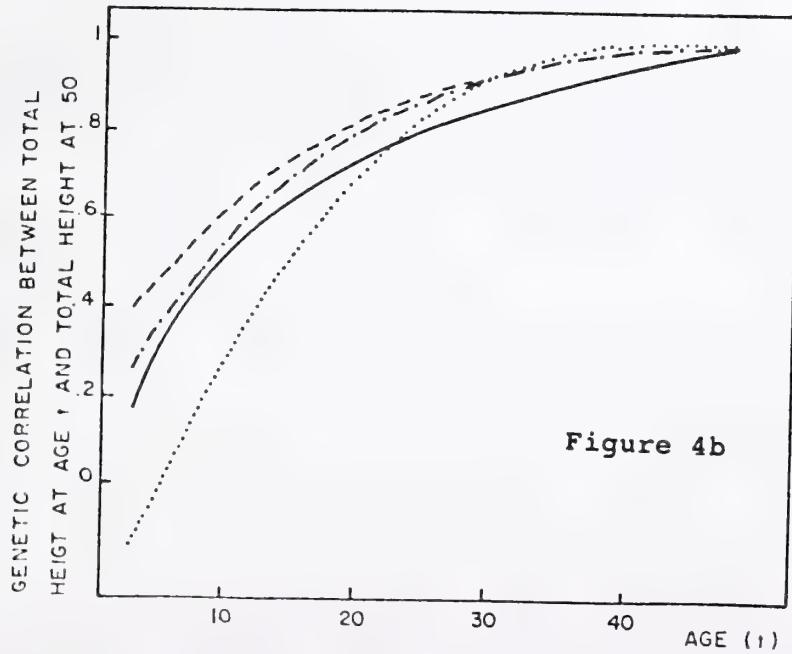
3. GENETIC ARCHITECTURE OF HEIGHT GROWTH AND IDENTIFICATION OF SELECTION CRITERIA.

Genetic control

First level of subdivision.

Additive variance of single AHIs represents generally between 60 to 90 % of the total genotypic variance. Heritability values of sums of successive AHIs increase with the number of increments reaching between .3 to .5 when several AHIs are summed. This variation is due to the decrease of the environmental variance (relative to the total phenotypic variance) when several succesive AHIs are added (Kremer, 1981b).

Second level of subdivision

Among all the components assessed PSL exhibits the highest heritability value. On six successive AHIs, the heritability values of AHI varied between .06 and .12, whereas corresponding values for PSL varied between .14 and .28 (Kremer and Lascoux, 1988). Heritability values of AESS and SSL are lower and more variable from year to year (Kremer and Lascoux, 1988). Additive variance of these traits represent from 60 to 90 % of the total genotypic variance.

Third level of subdivision

Compared to the other components NSU and MSUL are more affected by non additive gene effects. Additive variance of theses traits represent less than 40 % of the total genotypic variance reaching values of 20% for MSUL (Lascoux, 1984). As a result their heritability values are lower than corresponding values of other components.

Comparisons between the proportion of genotypic variance explained by additive variance provide pertinent information on gene effects controling height growth (table 1). Since NSU and MSUL are mostly due to non additive gene effects and are generally negatively correlated, and because their resulting trait (PSL) is mostly explained by additive effects, one may conclude on epistatic effects between genes of NSU and MSUL loci affecting primary shoot length.

Growing season	Height growth components		
	PSL	NSU	MSUL
9th season	56	28	18
10th season	74	37	12

Table 1 Ratio of additive variance on total genotypic variance for different height growth components (in percentage). These variances were estimated in a factorial

mating design (15 male parents and 15 female parents comprising 112 full sib families (Kremer and Lascoux, 1988).
Genetic correlations between components.

Components are never genetically independent. Refering to the different levels of subdivision , results are as follows:

First level of subdivision.
Successive AHIs are positively genetically correlated as shown on figure 3a; the correlation decrease with the lag separating AHIs.

Second level of subdivision.
PSL and AESS (and SSL) are generally negatively correlated (Kremer, 1981a; Kremer and Lascoux, 1988).

Third level of subdivision
The genetic correlation between NSU and MSUL varies, but are in most cases negative (Lascoux, 1984; Kremer and Lascoux, 1988).

Contribution of components to the composite traits.

First level of subdivision
Successive AHIs have similar contribution coefficients (Kremer, 1984) to the total height (Kremer and Lascoux, 1988). We are lacking data on comparisons between AHIs assessed during the juvenile stage and older AHIs.

Second level of subdivision
PSL shows systematically higher contribution to AHI than SSL: contribution coefficient values range between .7 and .8 for PSL and .2 to .3 for SSL (Kremer , 1984; Kremer and Lascoux, 1988).

Third level of subdivision
In most cases studied so far NSU has a larger contribution to PSL (or SSL): in general differences between contribution coefficient values are important (.8 for NSU versus .2 for MSUL) (Kremer, 1984). Interestingly results of age-age correlation relative to these morphogenetical components have shown that MSUL has a higher stability than NSU (Kremer and Li, 1989).

The analysis of genetic architecture of height growth shows that only PSL may be an alternative selection criteria for total height to AHI, mainly because of its higher heritability value (Boissieras, 1984; Kremer, 1988). Although we are lacking data on age-age correlation of components, there is a low chance that morphogenetical components (NSU or MSUL) will prove to be better early selection criteria, since they are mostly affected by non additive gene effects (table 1).
These results show also that there is an important genetic plasticity of height growth as revealed particularly by the variation of the genetic correlation between components indicating either compensation or cooperation between components (Kremer, 1984). Similar performances in height growth can be obtained by various pathways. As a result search

of early selection should take into account components *per se*
but also relationships between components or pathways followed
to reach the final level of height growth. Due to the
plasticity of height growth, expectations to identify single
component as early selection criteria are questionable. One
may find in specific studies that one component at a juvenile
stage exhibits a high JM correlation with total height in the
field (Kremer and Li, 1989). In this cited study MSUL in the
first season has shown the highest correlation with total
height at age 6. There is a definite repeatability problem: in
a different study another component may show a better
correlation. Several reasons may explain the discrepancy of
the results:

- sampling of families and seedlings in retrospective tests
  has a strong effect on JM correlation (Lambeth, 1983).
- genes controlling growth may have pleiotropic effects and
  alternatively affect different components.
- relationships between components may be modified in
  different environments. As stated earlier these
  relationships are to be taken into account in early
  evaluation studies.

The conclusion of the analysis of the "genetic architecture
of height growth" is that (i) only a multivariate approach
revealing expression of a great number of genes involved in
growth process would lead to the identification of potential
criteria (ii) the plasticity has an important impact on the
expression of growth; therefore more efforts should be devoted
to basic research on height growth based on explicative
approaches.

4. RESULTS FROM EXPLICATIVE APPROACHES: CHOICE OF TESTING
ENVIRONMENT.

The low juvenile mature correlation of height growth may be
due to several biological or genetic reasons:

(i) Existence of maternal effects. These have often been
attributed to the importance of the reserves contained in the
endosperm of conifer seeds, which is exclusively of maternal
origin.

(ii) Genotype-interaction effects. These can be attributed to
the differences between environmental conditions during the
juvenile stage (nursery environment) and the adult stage
(field or forest conditions), especially levels of drought.

(iii) Maturation effects. Height growth may be controlled by
different genes at different ages. This hypothesis would fit
with the serial correlation structure illustrated on figure
3a. The trend of variation of genetic correlation may be
intrepreted by a progressive change with age of the subset of
loci controlling the expression of height growth.

The first hypothesis was tested in a full diallel cross. The
6 parents of the mating scheme were of contrasting height
growth pattern, 3 expressed mainly several growth cycles at an

214

adult stage, and the 3 others only one cycle. Results obtained on the 5 first growing seasons showed that reciprocal effects were only significant during the first growing season (Guignard, 1983; table 2).

Growing season	Height growth component	GCA	SCA	GRE	SCE
1st	AHI	28.5**	2.0*	24.3**	6.6**
	NSU	12.6**	1.1	9.9**	2.4**
	MSUL	18.8**	2.0*	8.7**	6.2**
3rd	AHI	4.9**	1.4	.6	1.0
	NSU	5.5**	1.5	.6	.5
	MSUL	1.8	2.2**	1.0	.7
4th	AHI	6.0**	2.3*	1.7	.4
	NSU	9.4**	1.1	1.6	.7
	MSUL	5.4**	2.1*	2.2	1.1
5th	AHI	6.0**	1.9	2.6	1.6
	NSU	6.9**	1.1	1.1	1.0
	MSUL	6.4**	1.0	.8	.8

Table 2 F test values corresponding to the different sources of variation for height growth components.
* : F test significant at the 5% level
**: F test significant at the 1% level
GCA, SCA : General, specific combining ability
GRE, SRE : General, specific reciprocal effects
Data originated from a full diallel mating design with 6 parents (see text), data were not availiable for the second growing season.

The two other hypothesis were tested on a retrospective test comprising 18 open pollinated families. These were subdivided in 3 classes according to height growth performances of the parents (high, average and low performance). Each class was again subdivided in 2 subsets comprising families expressing mostly polycyclic shoots and monocyclic shoots. General combining abilities for height growth and polycyclism estimated in older progeny tests (between age 10 and 18) and clonal values estimated in grafted clonal tests were used as criteria to select the 18 parent trees of the retrospective progeny test.

Genotype*environment interaction

The 18 families were raised in the nursery in standard conditions during the two first growing seasons. At the end of the second growing season (August 30 to October 25) when the apical meristem was still initiating cataphylls, the material was separated in two treatments. The first consisted to induce drought by watering only with half of the water consumption of non stressed plants (treatment 2). The same treatments were again applied during the entire third season. Predawn water potential of stressed and non stressed plants was respectvely -.09 and -.04 MPa when the difference was maximum. There were no significant differences of water potential between families.

The trees formed up to 5 cycles, but significantly less in the stress treatment. No association between adult height growth class and length of the different cycles was found except for the first cycle (table 3) in the treatment where drought stress was applied. It is clear from that table that the stress treatment revealed a variation pattern corresponding to the different adult classes. Results of the non stress treatment did not reveal any family differences associated with adult height growth subdivision (Nguyen, unpublished data).

drought stress				no stress			
family	class a)	PSL b)	duncan grouping	family	class a)	PSL b)	duncan grouping
155	+	248		156	+	327	
156	+	243		150	0	318	
157	+	232		154	0	313	
158	+	231		136	–	290	
144	–	230		151	0	288	
142	0	221		155	+	280	
136	–	216		139	–	278	
151	0	211		157	+	277	
139	–	205		159	+	267	
150	0	204		142	0	258	
154	0	199		153	0	248	
147	–	194		144	–	247	
152	–	193		149	0	242	
149	0	186		158	+	228	
153	0	181		141	–	226	
148	+	171		147	–	226	
141	–	156		148	+	223	
159	+	144		152	–	197	

Table 3 Ranking of the families in two different treatments (with drought stress (S) and without (T))
a) height growth class according to adult performances (+ : top ranking families, 0 : mid ranking ; - : bottom ranking)
b) length of the first shoot of the third growing season (in millimeters)

Maturation

The 18 families were grown during their first season in two controlled environment rooms that differed in temperature and mainly in photoperiod. The experiments were conducted in the frame of a cooperation with the Swedisd University of Agricultural Sciences (The Phytotron, D. Ingegerd Dormling). Temperatures regimes were 25°C/20°C and 25°C/15°C during 16 and 8 hours respectively, and photoperiods were 24h and 16h. Seedlings raised in the continuous day treatment exhibited shorter duration of growth. 35% of the seedlings formed adult-like buds whereas seedlings in the discontinuous day treatment formed only rosettes. Adult-like buds were associated with a higher percentage of stem units bearing axillary short shoots (15% versus 7 % between the two treatments). Seedlings in the continuous day treatment exhibited morphological traits characterizing mature trees, particularly the development of secondary needles (Lascoux, unpublished data)

Eighteen traits related to growth of the seedlings were assessed during the first growing period: height growth curve parameters (4), growth components (3), morphological traits (5), above ground dry weights of different components (6). Among these traits, six showed significant differences between the adult height growth classes in the continuous day treatment. These traits reduced to only one in the discontinuous day treatment (table 4).

In the case of forest trees, accelerated maturation in controlled environments can induce the expression of genes that would normally be expressed only at an older stage. In this respect our results obtained in maturation experiments can be compared to other studies conducted in loblolly pine (Williams, 1987). In this study, height at the first bud was negatively correlated with eight-year height, whereas height accrued after first bud set was positively correlated. By inducing secondary needle development in continuous days we obtained already significant relation between components and later field growth.

The results obtained in the drought stress and the photoperiod experiments support the hypothesis that different loci are involved in different environments or at different ages. Interestingly there are now some experimental data based on QTL that have lead to the these conclusions in tomato (Paterson et al, 1991): among 29 QTLs underlying phenotypic variation, only 4 are detected in all three environments, other QTLs are specific to two or one environment only. Comparative QTL studies conducted in different environments or at different ages can therefore contribute to identify sets of loci specific to these conditions. As a consequence, one may use these results to identify optimal growing conditions during the juvenile phase for early evaluation. Therefore there is still a need of further research to identify optimal juvenile environmental conditions which reveal genetic differences associated to adult height growth performances.

These research should associated molecular approaches with morphophysiological approaches, which both are currently going on in maritime pine.

Component	Class		
	+ c)	0	−
Continuous light			
AHI (mm.)	175 a	196 b	181 a
NSU	250 a	279 b	269 b
DWG d)	118 a	168 b	176 b
UDWPRIM d)	.28 a	.28 a	.26 b
DWST d)	51 b	64 a	55 b
NSU PREF d)	72 a	79 b	77 b
Discontinuous light			
DWB d)	90 a	123 b	130 b

Table 4. Mean values of the different classes for various first season traits. Means are only represented for criteria showing significant differences between the classes.
c) height growth classes according to adult performances (+ : top ranking families; 0 : mid ranking ; − : low ranking).
d) DWB   : dry weight of branches at the base of the epicotyl on first season seedlings (10^{-2} gr.)
  UDWPRIM : mean dry weight of a primary needle (10^{-2} gr.)
  DWST   : dry weight of the stem (10^{-2} gr.)
  NSU PREF : number of primary needles preformed in the bud or the rosette.

Class means not significantly different (Tukey and LSD test at p=.05) are underlined with identical letters.

CONCLUSION

Results obtained so far have pratical implications in the genetic tree improvement program. First, if traditional technics are used (total height as selection criteria), selection can only be achieved when trees are older than 12 years. These results are obtained with experimental data and with simulations of age-age correlation. Selection at earlier stages requires to use different selection criteria, among which PSL may be an interesting alternative, provided it is assessed over several successive years.

Although some morphogenetic components (especially MSUL, Kremer and Li, 1989) in their first season are significantly correlated with later field performance, it is to early to decide on their definite use in practical breeding, because of the plasticity of height growth which can dramatically affect the repeatability of this first result obtained in one experiment. A first solution to the problem of repeatability is to use several components as selection criteria (multivariable approach).

As the analysis of genetic architecture of height growth and the explicative approaches have shown, the lack of JM correlation lies probably in the versatility of gene action at loci affecting growth:

- additive (for some components) versus non additive (for other components), epistatic effects between genes at loci controling NSU and MSUL.
- expression of regulation genes that can explain the plasticity of height growth.
- alternate sets of loci affecting growth at different ages or different environments

Therefore there is a need toward basic knowledge on genetic control of height growth. Molecular markers can be used to identify QTL involved in different situations (environments or stage of development). We expect that these analysis will lead, by comparing the different sets of loci, to the identification of optimal testing conditions at a juvenile stage, where early evaluation can be achieved with traditional compoments.

Literature cited

Baradat, Ph. and P. Pastuzka. 1990. Stratégie d'amélioration et diversification variétale du pin maritime.,p 375-390, in proceedings of 3eme colloque Sciences et industries du bois, " De la forêt cultivée à l'industrie de demain", Bordeaux, 14-15 Mai 1990

Boissieras, A. 1984. Recherches de prédicteurs juvéniles de l'aptitude génétique à la croissance en volume du pin maritime. Détermination de l'âge optimum pour la sélection. Mémoire de stage B.T.S. Option Forêts, Ecole forestière de Meymac, 58p.

Cannell, M.G.R. 1978. Components of conifer shoot growth. In : Proceedings of the fifth north american forest biology workshop. (C.A. Hollis and A.E. Squillace, eds) p.313-318, University of Florida, Gainesville.

Debazac, E.F. 1963. Morphologie et sexualité chez les pins. Rev. For. Fr. 114: 173-179

Doak, C.C. 1935. Evolution of foliar types, dwarf shoots, and cone scales of Pinus. Ill. Biol. Monogr. 13: 1-106

Guignard, P. 1983. Contrôle génétique du développement de semis de pin maritime (*Pinus pinaster* Ait.)). Mise en évidence des effets maternels sur la croissance au stade juvénile. Mémoire de D.E.A., Université de Bordeaux 58p.

Illy, G. 1966. Recherches sur l'amélioration génétique du pin maritime. Ann. Sci. Forest. 23: 757-948

Kremer, A. 1981a. Déterminisme génétique de la croissance en hauteur du pin maritime (*Pinus pinaster* Ait.). 1. Rôle du polycyclisme. Ann. Sci. For. 38: 192-222

Kremer, A. 1981b. Déterminisme génétique de la croissance en hauteur du pin maritime (*Pinus pinaster* Ait.). 3. Evolutiondes composantes de la variance phénotypique et génotypique. Ann. Sci. For. 38: 355-375

Kremer, A. 1984. Component analysis of height growth, compensation between components and seasonal stability of shoot elongation in maritime pine (*Pinus pinaster* Ait.). In "Crop physiology of forest trees" (P.M.A. Tigerstdet, P. Puttonen, V. Koski eds.), Helsinky University Press, p 203-217.

Kremer, A. and D.M. Lascoux. 1988. Genetic architecture of height growth in maritime pine (*Pinus pinaster Ait.*). Silvae genetica 37: 1-8

Kremer, A. and Li An Xu. 1989. Relationship between first-season free growth components and later field height growth in maritime pine (*Pinus pinaster* Ait.). Can. J. For. Res. 19: 690-699

Lambeth, C.C. 1980. Juvenile-mature correlations in Pinaceae and implications for early selection. For. Sci. 26: 571-580

Lambeth, C.C. 1983. Early testing overview with emphasis on loblolly pine. In Proc. 17th. South. For. Tree Improv. Conf. p 297-311

Lascoux, D.M. 1984. Décomposition de la croissance en hauteur du pin maritime. Aspects morphogénétiques et génétiques. Mémoire de fin d'études à l'ENITEF, INRA, Cestas, 80p

Lemoine, B. 1981. Application de l'analyse factorielle à l'étude de la croissance en hauteur des arbres: exemple du pin maritime. Ann. Sci. For. 38: 31-54

Patterson, A.H., S. Damon, J.D. Hewitt, D. Zamir, H.D. Rabinowitch, S.E. Lincoln, E.S. Lander, and S.D. Tanksley 1991 Mendelian factors underlying quantitative traits in tomato: comparison across species, generations, and environments. Genetics 127: 181-197

Williams, C.G. 1987. The influence of shoot ontogeny on juvenile-mature correlations in loblolly pine. For. Sci 33: 411-422

VERIFICATION TRIAL FOR EARLY SELECTION OF LOBLOLLY PINE

S. E. McKeand[1]
F.E. Bridgwater[2]

Abstract.--In previous research, stem elongation in first-and second-year loblolly pine (Pinus taeda L.) seedlings has reliably predicted 8- to 12-year heights in eastern NC and SC provenances. However, it is possible that stem elongation traits will not be reliable for early selection with other provenances. In the Western Gulf Cooperative, total stem dry weight at 4-6 months is used to predict field performance, but dry weight has not been a good predictor in studies of the eastern NC provenance.

A study was established in southwest Georgia with 13 to 16 OP families from each of five provenances to test differences among provenances for early selection. Ranks of heights and stem elongation traits for the five provenances were as expected, and heritabilities for stem elongation traits were moderate to high. Heights and elongation traits were strongly related to 5-year heights in older trials for the Atlantic Coastal and Middle-Upper Gulf Provenances, but the relationships were weaker for the Lower Gulf, Marion Co., FL and Gulf Hammock, FL provenances. There is evidence that early selection based on first- and second-year stem elongation traits will be effective only for certain provenances of loblolly pine. To verify these preliminary results with only five-year "mature" data, the older field trials will be measured through rotation and the long-term relationships determined.

[1] Cooperative Tree Improvement Program, N.C. State University, Raleigh, NC
[2] U.S. Forest Service, Southeastern Forest Experiment Station, Raleigh, NC

A GEOGRAPHIC VARIATION STUDY OF FRASER FIR

J. B. Jett, S. E. McKeand, and Yecei Liu
North Carolina State University
Raleigh, NC

Open-pollinated seeds were collected from 10 trees in elevational stands (provenances) in five major natural populations of Fraser fir (Abies fraseri (Pursch) Poir). Field trials were established at three locations in western North Carolina with 1-2 seedlings. Total height, crown diameter, branch diameter, number of buds in the terminal whorl (terminal buds), and number of buds on a first-order lateral branch (branch buds) were measured after four growing seasons in the field. An estimate of crown density was derived by multiplying terminal buds by branch buds.

There were significant differences ($p \leq 0.05$) among provenances for all traits measured in this study. Low elevation provenances (1500m and 1650m) tended to out-perform the high elevation provenances (1800m and 1950m). The two provenances most frequently used for commercial seed collection of Fraser fir, Roan Mountain-1650m and 1800m, were significantly poorer than the best provenances for height, crown diameter, and density. Provenance x location interactions were significant for height, crown diameter, terminal buds, and branch diameter. Significant family x location interactions were found for density, terminal buds, and branch buds.

Estimates of individual tree heritabilities indicated that most of the important traits were under moderate to strong genetic control (height = 0.42, crown diameter = 0.32, density = 0.34, terminal buds = 0.13, branch buds = 0.41, and branch diameter = 0.28), and selection should result in large genetic gains. Strong genetic correlations between height and crown diameter (0.89), density (.69), branch diameter (0.86), and branch buds (0.73) indicate that improvement for growth rate will also result in improvement of traits that are related to Christmas tree grade.

GENE TRANSFER IN CONIFERS

A.-M. Stomp, D. Robertson[a], A.K. Weissinger[a] and R. Sederoff

Departments of Forestry & Crop Science[a],
North Carolina State University
Raleigh, NC

Conifers are a major group of crop plants, accounting for much of the World's supply of pulp and solid and composite wood products. Genetic improvement is slow due to the long breeding and testing cycles for these plants. If methods are developed specific for conifers, the tools of molecular biology have the potential to accelerate genetic improvement. One of the critical tools under development in our laboratories is gene transfer. We report here our progress in using the biolistic microprojectile DNA transfer system to transform three conifer species: loblolly pine, norway spruce and Fraser fir. The target tissue and culturing system used with each species is different. The meristematic tissue produced when cotyledons are incubated on cytokinin containing medium is being used with loblolly pine (see accompanying poster abstract). Bombardment of somatic embryos is being used to explore transformation in norway spruce. Both of these systems have yielded stably transformed cell cultures. Experiments are in progress to obtain transgenic plants. Work with Fraser fir has only begun and we have shown transient expression in bombarded expanding apical meristems. This presentation will focus on the strategy for obtaining transgenic conifers using microprojectile bombardment.

EARLY FIELD PERFORMANCE OF RUST-RESISTANT CLONES OF SLASH PINE:
A COMBINATION OF DIRECT AND INDIRECT SELECTION

J. L. Ford-Logan [1], G. S. Foster [1], and J. P. van Buijtenen [2]

Abstract.--Two-month old slash pine (Pinus elliottii Engelm. var. elliottii) seedlings from four known rust-resistant, full-sib families and three known rust-resistant, open-pollinated families were subjected to a standardized fusiform rust inoculation test. Based on rust infection readings taken 4 months post-inoculation, all the families showed greater resistance than the susceptible checklot (GA SL). Five of the seven families showed greater resistance than the resistant checklot (FA2), based on their Index of Relative Resistance.

In a field study conducted near Bogalusa, Louisiana, all seedlings without galls were cloned by rooted cuttings and outplanted, along with two seedling checklots. After two growing seasons, survival averaged 95% for the rooted cuttings and 98% for the seedlings. Rust infection was 1% for the rooted cuttings compared with 43% and 29% for the resistant and susceptible seedling checklots, respectively. The rooted cuttings were smaller than the seedlings after one growing season (0.83 and 1.07 ft, respectively), but this difference can probably be attributed to differences in initial planting size. During the second growing season, both propagule types grew a similar amount in height (1.51 ft for rooted cuttings and 1.56 ft for seedlings).

Keywords: fusiform rust, Pinus elliottii Engelm. var. elliottii, rooted cuttings, rust resistance, seedlings.

INTRODUCTION

Fusiform rust, caused by Cronartium quercuum (Berk.) Miyabe ex Shirai f. sp. fusiforme, is a serious disease affecting slash pines (Pinus elliotii Engelm. var. elliottii) and other pines over much of the southeastern United States (Powers et al., 1975; Anderson et al., 1986). Tree improvement programs using genetic resistance in slash pine populations along with clonal forestry techniques offer significant promise for reducing losses caused by this devastating fungus in commercial timber plantations.

Clonal forestry is becoming increasingly important as a reforestation tool, with clonal propagation providing a means of exploiting both the additive genetic gain achieved through sexual breeding and the nonadditive genetic gain identified through testing for superior genotypes. Predicted gains from vegetative propagation techniques, however, have been based on the assumption that cuttings will perform comparably with seedlings. If cuttings do not grow as well as seedlings, predicted genetic benefits of rooted cutting programs will not be realized. Comparative field tests of rooted cuttings and seedlings from genetically related populations should provide the information necessary to assess the potential of vegetative propagules in clonal reforestation and tree improvement programs. Used together, artificial

---

[1] Plant Physiologist and Research Geneticist, respectively, USDA Forest Service, Southern Forest Experiment Station, Alabama A&M University, P. O. Box 1208, Normal, Alabama 35762.

[2] Head of Reforestation Department, Texas Forest Service, and Professor, Forest Science Department, Texas Agricultural Experiment Station, Texas A&M University, College Station, Texas 77843.

inoculation procedures and rooted cutting techniques may provide a powerful tool for increasing selection effectiveness and reducing the time required to enhance rust resistance in pine stands significantly.

Foster and Shaw (1987) proposed a program in which known disease-resistant parents (direct selection) of loblolly pine (Pinus taeda L.) would be mated and their seedling offspring subjected to an initial screening for disease resistance (indirect selection). The resistant seedlings would then be cloned and the clones planted in field trials. After a few years, the most resistant clones would be selected (direct selection) for further use in a large scale reforestation program. A subsequent study by Foster and Anderson (1989) tested the feasibility of such an approach. The resulting tree improvement program was effective in developing genotypes of loblolly pine that were highly resistant to the fusiform rust fungus, and preliminary evidence suggested that the program could be effective in providing highly rust-resistant clones for reforestation.

The objectives of this study were (1) to test the effectiveness of direct and indirect selection for fusiform rust resistance in slash pine seedlings of which the gall-free survivors were cloned and established in a field trial, and (2) to estimate genetic parameters for height growth from a clonal study with slash pine.

MATERIALS AND METHODS

Slash pine seeds for this experiment were obtained from the Texas Forest Service and originated from three open-pollinated and four full-sib family seedlots (Table 1). The parents for all families were selected for superior resistance to fusiform rust based on field progeny test results. Seedlings were grown and inoculated with fusiform rust spores via a standard rust-screening process used in the U.S. Forest Service Resistance Screening Center (Anderson et al., 1983).

Table 1. Rust data for the slash pine open-pollinated and full-sib families and number of clones per family in the field study.

Family	Entry code	Resistance Screening		Clones in field test
		Index[1]	% galled	
D5PC98	1	194.00	52.3	3
D5PC286	2	136.66	56.3	7
S4PC1	3	160.08	73.2	3
D4PC13 x D5PC268	4	246.46	37.7	5
D4PC13 x S5PC3	5	166.02	41.8	4
D4PC40 x D5PC286	6	229.24	33.8	5
S4PC5 x S5PC5	7	140.26	62.2	2
GA SL [2]		83.14	76.3	
FA2 [3]		143.10	68.3	

[1] Index from Walkinshaw et al. (1980).
[2] Resistance Screening Center susceptible standard checklot.
[3] Resistance Screening Center resistant standard checklot.

Gall-free seedlings were cloned at 6 months of age for use in a field trial. The seedlings were shipped to Bogalusa, Louisiana and uppotted to 3-gallon pots in September 1984. Terminal buds were removed from each seedling shortly after uppotting to enhance shoot production and delay further height growth. The trees were maintained outside in a shadeframe. In May 1985, 4-inch long cuttings were collected from each seedling and were set for rooting. The cuttings were rooted by the International Forest Seed Company of Odenville, Alabama using their standard procedures (Hughes, 1987).

The rooted cuttings were received from International Forest Seed Company in late fall 1985 and were prepared for field planting. Since slash pine has little or no chilling requirement, the rooted cuttings were placed immediately in a greenhouse and grown using normal watering and fertilization procedures. The rooted cuttings were grown in 10-in^3 Leach Super Cells^{TM}. Seedlings representing two checklots were also grown in the greenhouse in similar containers. One seedlot (BRCK) came from a first-generation seed orchard owned by the Brunswick Pulp Land Company of Brunswick, Georgia and included genetically superior trees selected for growth and rust resistance. The parent trees originated in south Georgia and north Florida. The other seedlot (WGCK) was a commercial checklot from a seed production area in southeast Texas and developed by the Western Gulf Forest Tree Improvement Program for use in their progeny tests, and could be expected to show some genetic improvement.

By spring 1986 the rooted cuttings and seedlings were ready for field planting. They were planted near Bogalusa, Louisiana on May 12, 1986 at an 8 x 8-ft spacing. Two to four (mainly three) ramets per clone and twelve seedlings per seedling checklot were planted in each of two replications in a randomized complete block design. This yielded a total of four to seven (mainly six) ramets per clone and 24 seedlings per checklot. The ramets per clone or seedlings per checklot were randomized within each replication in a noncontiguous fashion.

Measurements were recorded at the end of the first and second growing seasons, and then the study was accidently burned, thereby destroying it. Total height was measured at years one and two, and presence of a rust gall was observed at year two. All galls were located on the main stem; therefore gall location data were unnecessary.

Analysis of variance of height data was computed using a model that included clones, replications, clone x replication interaction, and within-plot sources of variation (Table 2). The two seedling checklots were not included in this genetic analysis. Variation by family source was not measured because there were only two family types (full-sib and open-pollinated) and there was a small number of families per type. With family types combined, a total of 29 clones were included in the analysis. All sources of variation were considered to be random. Variance components were calculated by equating the mean squares and expected mean squares (type III, PROC GLM; SAS Institute Inc., 1985).

Table 2.   Form of the analysis of variance for analyzing height growth data of 29 slash pine clones.

Source of variation	Degrees of freedom	Expected mean squares[1]
Clones (C)	28	$\partial^2 + 2.7 \partial^2_{CR} + 5.3 \partial^2_C$
Replications (R)	1	$\partial^2 + 2.5 \partial^2_{CR} + 72.6 \partial^2_R$
C x R	28	$\partial^2 + 2.7 \partial^2_{CR}$
Within-plot	98	∂^2
Total	155	

[1] Symbols explained in text.

   Broad-sense heritabilities were calculated on an individual-ramet basis (H^2) and on a clone-mean basis (H^2_x). Selection of clones is generally based on their average performance, hence the second

heritability is most appropriate.

$$H^2 = \frac{\partial^2_C}{\partial^2_C + \partial^2_{RC} + \partial^2} \tag{1}$$

$$H^2_x = \frac{\partial^2_C}{\partial^2_C + \dfrac{\partial^2 RC}{r} + \dfrac{\partial^2}{nr}} \tag{2}$$

where

∂^2_C = variance among clones,

∂^2_{RC} = variance due to interaction between clones and replications,

∂^2 = variance among ramets within a clone and replication plot,

r = number of replications,

n = number of ramets per clone and replication.

The rust data were not subjected to statistical analysis since virtually no rooted cuttings were infected by the rust, whereas substantial numbers of both seedling checklots were infected. Most of the clones would therefore have zero values for percentage of trees with galls, while the seedlings displayed more traditional distributions of rust data values.   We concluded that assumptions for analysis of variance (normal distribution of errors and equality of variances among treatments) could not be met even with data transformation.

The late spring (May 12, 1986) planting of the rooted cuttings and seedlings resulted in a decrease in average height at the end of the first growing season (0.83 ft for rooted cuttings and 1.07 ft for seedlings) compared with potential first-year growth of slash pine when planted during the winter. The difference in height between propagule types was more an effect of initial planting size (i.e., rooted cuttings were shorter than seedlings) than differential growth. Although height growth during the first season was low for both propagule types, survival of the trees was very high, with 95% of the rooted cuttings and 98% of the seedlings surviving. First-year average height of the 29 clones ranged from 0.58 to 1.06 ft, while the two seedling checklots averaged 1.17 ft (BRCK) and 0.97 ft (WGCK).

Analysis of variance of first-year height (HT1) of rooted cuttings revealed no significant difference among clones (Table 3). The clonal source of variation accounted for 8% of the total variation. Broad-sense heritability on an individual basis was low ($H^2 = 0.08$), indicating low genetic control of the trait. However, once heritability was expressed on a clone-mean basis, it increased fourfold to $H^2_x = 0.31$.

Micro-site effects or random propagation effects were probably the cause of low heritabilities.

Table 3. Mean squares and variance components for height at ages one and two in a clonal test of slash pine.

Source of variation	Height 1			Height 2		
	Mean squares	Variance components		Mean squares	Variance components	
		value	% total		value	% total
Clones (C)	.0700*	.0047	8	.6538+	.0598	12
Replications (R)	.0237*	< 0	0	.4844*	.0020	1
C x R	.0449*	< 0	0	.3369*	< 0	0
Within-plot	.0564	.0564	92	.4275	.4275	87

* Not significant at p = 0.05.
+ Significant at p = 0.05.

The rooted cuttings and seedlings appeared to have become acclimated to their environment by the second growing season. Second-year height (HT2) averaged 2.34 ft for rooted cuttings and 2.63 ft for the seedlings; hence the growth increment during the second growing season was almost identical for rooted cuttings (1.51 ft) and seedlings (1.56 ft). The HT2 ranged from 1.53 to 2.91 ft for the clones and averaged 2.73 ft for BRCK and 2.53 ft for WGCK. The slightly inferior HT2 of the clones versus the seedling checklots is somewhat disappointing, especially compared with the WGCK checklot; however, the fact that the WGCK checklot is slightly genetically improved would lessen the expected difference in growth. We hypothesize that smaller planting size of the rooted cuttings continues to influence size differences at 2 years of age. The genetically superior rooted cuttings might have overtaken the WGCK checklot seedlings if the trees had survived to an older age, but there was no opportunity to test this hypothesis in the current study since they were destroyed.

The genetic analysis of height growth of the clonal portion of this study at age two is one of the largest contributions of this study. There is a distinct lack of such information in the scientific literature for slash pine. The clonal variance is significant for HT2, and this source accounts for 12% of the total variation (Table 3). Genetic control of HT2 increases, compared with HT1, as evidenced by a larger

broad-sense heritability ($H^2 = 0.12$). Clonal selection should be more effective at age two than at age one since $H^2_x = 0.43$, which is substantially greater than mean HT1.

Even though the mean HT2 of the clones was somewhat lower than that of either of the two checklots, selection of the superior clones will lead to a reasonable level of realized genetic gain on similar sites. The average HT2 of the 10% of the clones (2.87 ft) exceeds the HT2 of the WGCK checklot by 13% (2.87 vs 2.53 ft) and of the BRCK checklot by 5% (2.87 vs 2.73 ft).

The enhanced resistance to fusiform rust of the rooted cuttings versus the checklots is unequivocal. The select clones averaged only 1% rust infection at age two compared with 43% for BRCK and 29% for WGCK (Table 4). Of the 29 clones in the study, only two became infected; and in both cases a single ramet out of five or six ramets per clone was affected.

Table 4. Field rust infection data at age 2 years for 29 clones and two seedling checklots expressed as percent galled (either ramets per clone or seedlings per checklot).

Entry[1] code	Percent galled							
	Clones within family							
	1	2	3	4	5	6	7	Seedlot
1	0	0	0	0	0	NA	NA	
2	0	0	NA	NA	NA	NA	NA	
3	0	0	0	NA	NA	NA	NA	
4	0	17	0	0	NA	NA	NA	
5	0	0	0	20	0	NA	NA	
6	0	0	0	NA	NA	NA	NA	
7	0	0	0	0	0	0	0	
BRCK								43
WGCK								29

[1] From Table 1 and text (for checklots).
NA not applicable.

At least two hypotheses exist for the high level of rust resistance in the select clones. First, the combination of direct selection of superior parents and indirect selection of seedlings followed by cloning may provide a highly effective genetic improvement strategy for fusiform rust resistance. Alternatively, the selection system may be strongly augmented by rust resistance due to maturation.

Support for the efficient genetic selection alternative comes from other genetic studies with slash pine. Unfortunately, results from other clonal studies regarding rust resistance of slash pine are not available in the scientific literature; hence seedling studies must be cited. Fusiform rust resistance in slash pine appears to be under moderate additive genetic control (Layton, 1985), and there is evidence of non-additive genetic control (Layton, 1985; Sluder, 1989). In a loblolly pine study, Foster and Anderson (1989) had results very similar (almost complete resistance of rooted cuttings) to those of this study. One difference in the studies was that the loblolly pine test was conducted under artificial inoculation at the Resistance Screening Center as compared with the field test in the current study. Researchers have found the Resistance Screening Center's artificial inoculation system to be particularly effective with slash pine (Walkinshaw et al., 1980), possibly more so than with loblolly pine (Sluder and Powers, 1986). We are concerned, however, that the narrow-sense heritabilities from other studies are not high enough to

explain our results. Rust resistance would have to be conditioned largely by non-additive genes in order to confirm our results under the efficient genetic selection alternative, and the few supporting studies do not confirm this.

Some evidence lends credence to the second alternative that genetic resistance is strongly augmented by maturation-induced resistance due to the propagation system. McKeand (1985) reported an unexpected increase in apparent level of maturation in a study with tissue-culture plantlets of loblolly pine even though the source tissue (cotyledons) was very juvenile. Rust resistance, for example, seemed to be enhanced in the plantlets versus seedlings from the same families. Similar evidence for either loblolly or slash pine rooted cuttings is lacking in the scientific literature. As clearly shown by Franklin (1969), Greenwood (1984), and Sweet (1973), among others, trees do mature; and trait expression changes as level of maturation changes. It has been recognized that resistance of white pines (Pinus monticola) to blister rust (Cronartium ribicola J. C. Fisch. ex Rabenh.) increases directly with host age, at least up to the age of 4 years (Bingham, 1969). The source of the slash pine cuttings in the current study was 17-month old seedlings. In a study with rooted cuttings from similar-aged loblolly pine, Foster (1988) showed no difference between growth and morphology of rooted cuttings and that of seedlings from the same families. Rust resistance data were not reported. In the current study, increased maturation of the rooted cuttings is one possible explanation for the slightly depressed growth of the rooted cuttings compared with seedlings; however, the two propagule types were not closely matched for initial size or genetic source. Therefore, close comparisons should not be made.

CONCLUSIONS

Enhanced maturation is one explanation for the large difference in rust resistance for the two propagule sources, although related studies do not provide much support for this hypothesis. A study should be conducted specifically to address this issue for rooted cuttings.

Rooted cuttings of slash pine appear to have promise as planting stock for reforestation. The selection system, combining direct and indirect selection, for choosing superior clones resulted in planting stock with superior field rust resistance. The exact mechanism, genetic versus maturation, behind this resistance is unknown; yet as long as the rooted cuttings grow normally otherwise, knowing the exact mechanism is mainly of academic interest. Early height growth is moderately controlled by genetic factors, thus leading to the possibility of reasonable levels of genetic gain by selecting superior clones.

ACKNOWLEDGEMENTS

LITERATURE CITED

Anderson,R.L., J.P.McClure, N.Cost, and R.J.Uhler. 1986. Estimating fusiform rust losses in five Southeast states. South. J. Appl. For. 10:237-240.

Anderson,R.L., C.H.Young, and J.D.Triplett. 1983. Resistance screening center procedures manual: a step-by-step guide used in the operational screening of southern pines for resistance to fusiform rust. (Revised June 1983). USDA For. Ser. State Priv. For. Rep. No. 83-1-18.

Bingham,R.T. 1969. Artificial inoculation of large numbers of *Pinus monticola* with *Cronartium ribicola*. In Biology of Rust Resistance in Forest Trees. p. 357-372 in Proc. of a NATO-IUFRO Advanced Study Institute. Moscow, ID.

Foster,G.S. 1988. Growth and morphology of rooted cuttings and seedlings of loblolly pine and their genetic analysis. p. 67-78 in Proc. of 10th North American Forest Biology Workshop. Vancouver, British Columbia.

Foster,G.S., and R.L.Anderson. 1989. Indirect selection and clonal propagation of loblolly pine seedlings enhance resistance to fusiform rust. Can. J. For. Res. 19:534-537.

Foster,G.S. and D.V.Shaw. 1987. A tree improvement program to develop clones of loblolly pine for reforestation. p. 17-21 in Proc. 19th South. For. Tree Improv. Conf. College Station, TX.

Franklin,E.C. 1969. Ortet age has strong influence on growth of vegetative propagules of *Pinus elliottii* Englem. Second World Consultation on Forest Tree Breeding. 8 p.

Greenwood,M.S. 1984. Phase change in loblolly pine: shoot development as a function of age. Physiol. Plant. 61:518-522.

Hughes,H.F. 1987. Cutting propagation of rust resistant hedges of *Pinus taeda*. Plant Propag. 1:4-6.

Layton,P.A. 1985. Genetic variation in symptomology of slash pine in response to fusiform rust. Ph.D. Thesis University of Florida, Gainesville.

McKeand,S.E. 1985. Expression of mature characteristics by tissue culture plantlets derived from embryos of loblolly pine. J. Amer. Soc. Hort. Sci. 110:619-623.

Powers,H.R.,Jr., J.P.McClure, H.A.Knight, and G.F.Dutrow. 1975. Fusiform rust: forest survey incidence data and financial impact in the south. USDA For. Ser. Res. Pap. SE-127.

SAS Institute Inc. 1985. SAS user's guide: statistics, version 5. SAS Institute Inc., Cary, NC.

Sluder,E.R. 1989. Fusiform rust in half-diallel cross progenies of resistant or susceptible loblolly and slash pines. p. 338-345 in Proc. 20th South. For. Tree Improv. Conf. Charleston, SC.

Sluder,E.R. and H.R.Powers,Jr. 1986. Further comparisons between infection of loblolly and slash pines by fusiform rust after artificial inoculation or planting. USDA For. Ser. Res. Pap. SE-342.

Sweet,G.B. 1973. The effect of maturation on the growth and form of vegetative propagules of radiata pine. N. Z. J. For. Sci. 3:191-210.

Walkinshaw,C.H., T.R.Dell, and S. D.Hubbard. 1980. Predicting field performance of slash pine families from inoculated greenhouse seedlings. USDA For. Ser. Res. Pap. SO-160.

Variation in the Wood Properties of the <u>Pinus elliottii</u> x
<u>Pinus caribaea</u> var. <u>hondurensis</u> F_1 Hybrid, Its Parental Species, and
Backcross to <u>Pinus elliottii</u> in Australia

D. L. Rockwood, K. J. Harding, and D. G. Nikles 1/

   Abstract.--The <u>Pinus elliottii</u> (E) x <u>Pinus caribaea</u> var.
<u>hondurensis</u> (H) F_1 hybrid (EH) has replaced improved E and improved
H on poorly-drained and well-drained sites, respectively, in
subtropical southeast Queensland due to its overall superiority. EH
is being evaluated in the subtropics world-wide, including Florida.
Due to its potentially greater frost-hardiness and only slightly
lower productivity, the EH backcross to E (BC) may be of value in
Florida and the coastal southeastern United States. Critical wood
properties and bark thickness of EH, associated E and H parents, and
BC were assessed in 21-year-old and 15-year-old studies at four
locations. EH wood properties may be evaluated by the first five
rings. Wood density and latewood percent tended to be greater at
poorer sites and also increased with proximity to the equator. Taxa
comparisons for density and latewood were E > BC > EH > H
consistently across locations; for extractives content, EH = H ≥ BC
≥ E with considerable location effect; and for heartwood percent,
minor differences. Extractives in EH and H heartwood generally
exceeded that of E. Large family differences within taxa typically
surpassed taxa differences. Taxa and families maintained their
rankings for wood properties across locations in the study with 10
families per taxon, but taxa ranks changed in the study with only
four families per taxon. Superior growth and generally acceptable
wood properties of EH favor its use over the pure species in
appropriate areas of the subtropics. Parental selection, followed
by EH evaluation, will insure wood properties comparable to E.
Commercial seed production and experimental vegetative propagation
practices are established at the Queensland Forest Service (QFS).

Keywords: <u>Pinus elliottii</u>, <u>Pinus caribaea</u> var. <u>hondurensis</u>, hybrid,
wood density, extractives content, latewood.

INTRODUCTION

   Recent exotic pine planting in Queensland favored E on swampy sites in the
southeast and H on naturally well-drained sites. Beginning with trials in 1958,
the F_1 hybrid between the two species demonstrated excellent growth and stem,

branch, and wood properties (Nikles et al. 1987), and in 1985, EH F_2 replaced E in commercial planting programs of the QFS, but H continued to be routinely planted on better drained central and southern coastal Queensland locations and on all northern Queensland locations (Last 1990). Seed orchard programs are in place to produce EH F_1 planting stock (Nikles and Robinson 1989), which will gradually replace all H and EH F_2 for operational planting in southern coastal Queensland (Nikles 1991). EH is of interest world-wide (Nikles et al. 1987, Nikles 1991), and evaluation trials have begun in Florida. Due to its potentially greater frost-hardiness, the BC may be of value in Florida and the coastal southeastern United States.

Considerable information exists on the wood properties of the parental taxa (e.g., Smith 1977), but only initial data are available for EH (Harding and Eccles 1990, Harding and Hagan 1990). Preliminary conclusions of several wood quality studies completed since 1971 by the QFS with E, H, and EH are: 1) EH is intermediate for density and latewood, 2) EH is similar for other wood properties, 3) EH sawn graded recovery is greater than E and relatively unimproved H (Bragg 1990), 4) EH resin % and defect incidence is similar to H, and 5) Between family variability exceeds between taxa differences.

For basic density, extractives content, heartwood percent, latewood percent, and bark thickness of EH, associated parents, and BC, this study had six objectives: 1) Investigate age-age relationships, 2) Confirm earlier indications of location effects, 3) Extend taxa by location interaction evaluations, 4) Broaden E, H, and EH comparisons, 5) Develop guidelines for predicting hybrid combining ability of E parents, and 6) Assess the potential of BC.

MATERIAL AND METHODS

Study 364, which contributed to Objectives 1 through 3, was planted near Whiporie, New South Wales, in 1969 and in 1970 at three Queensland locations (Table 1) with different soil and drainage characteristics. Open-pollinated (o-p) families of four E clones used as females for EH, o-p families of four H clones used as males for EH, and four EH families between these E and H clones were sampled to characterize the genetic growth range in their respective taxon. These families occurred within 60-tree noncontiguous plots at 3 x 2.7 m spacing. Stratified random sampling was used to select six trees, two trees from the lower-, mid-, and upper-1/3 of the DBH range, of each of the 12 families. Wood sampling consisted of 5 mm bark-to-bark cores taken at 1.0 to 1.2 m. Each tree's DBHOB, total height, and bark thickness were also determined. Volume inside bark was calculated by formulae appropriate for the taxa (Vanclay and Shepherd 1983, P. Gordon pers. comm.).

Study 464, which contributed to all objectives, was established in April 1976 at three Queensland locations, swampy sites with a range of productivity, and in June 1976 on a well drained site near Whiporie (Table 1). Ten E clones with o-p families also served as female parents for EH hybrids with a Beerburrum H pollen mix and a Byfield H pollen mix and for backcrosses with a Beerburrum EH pollen mix. A Beerburrum H bulklot (H_{Be}) and a Byfield H bulklot (H_{By}) represented the H parental populations. These genetic entries were allocated to 49- (7 x 7) or 80-tree (8 x 10) noncontiguous plots at 3 x 3 m spacing. Tree selection and wood sampling was as in Study 364. The E parents 1008 and 1011 and

234

Table 1. Summary of sampling by taxa and locations in Studies 364 and 464.

Taxon	Number of Sample Trees				Total
364 Location:	Whiporie (Wh)	Toorbul (To)	Coochin (Co)	Byfield (By)	
E	12	24	24		60
EH	12	24	24	24	84
H	—	24	24	18	66
Total	24	72	72	42	210
464 Location:	Whiporie	Husseys (Hu)	Tuan (Tu)	Byfield	
E	18	60	24	24	126
BC	18	60	21	24	123
EH_{Be}	18	60	24	24	126
EH_{By}		60			60
H_{Be}		30	24	24	78
H_{By}	—	30	—	—	30
Total	54	300	93	96	543

12 of the trees in the H_{Be} subsamples in Study 464 provided links to the larger subsamples and to Study 364.

Location and across-location effects were examined in subsets of genetic entries in 464-Tuan, 464-Byfield, and 464-Whiporie. Each subset taken in the field involved four E parents and 24 trees from the H_{Be} population, except in 464-Whiporie (Table 1). Studies 364 and 464 also assessed age-age relationships.

Field tree selection and wood sampling started in January 1991 and was completed in mid-April; laboratory analysis commenced as the wood samples were received. All cores were sectioned at the pith and planed on one surface to facilitate accurate ring count. Each radial core was marked inward from the cambium to denote the outer latewood boundaries of the annual rings. Then, the 5-, 10-, 15-, and 19-th rings from the pith in Study 364, and the 5-, 10-, and 13-th rings in Study 464, were located and their radial distances from the pith recorded, along with the radial distance to the outer extent of heartwood. Latewood percentage for E, EH, and BC families derived from female parents 1008 and 1011 in Study 464 was estimated as a proportion of radial distance by linear measurement of latewood using a 10x eyepiece graticule. Study 364 cores were subsequently sectioned into segments representing 0-5, 6-10, 11-15, and 16-19 years from the pith, with Study 464 core segments for 0-5, 6-10, and 11-13.

The segments were processed for unextracted wood density, moisture content, extractives content, and extracted wood density. Segments were submersed in water in a vacuum dessicator until saturated and then weighed in water and air. After oven-drying at 102°C for 16 hours, they were reweighed to derive unextracted wood density and moisture content. Extraction involved first methanol in a soxhlet apparatus for six hours followed by a further 8 hours in hot water. After 16 hours of oven-drying at 102°C and reweighing, extractives content and extracted wood density were calculated.

Combined core segmental values weighted by radial distance squared were the basis for correlating 5-, 10-, 15-, and 19-ring wood properties in Study 364 and 5-, 10-, and 13-ring properties in Study 464.

For the taxa, location effects and interaction across locations were assessed by various analyses of variance. Family comparisons in within taxa analyses and taxa comparisons in the combined taxa analyses were conducted by Duncan's Multiple Range Test. All analyses were done by SAS.

RESULTS AND DISCUSSION

Growth

The EH hybrids in the wood sample were larger than the parental taxa in almost all study locations (Table 2). After 21 years in Study 364, EH families had up to 50% more volume inside bark than the taxon previously preferred at each location. At 15 years of age in Study 464, the better EH taxon also had a similar advantage over the standard parental taxon at each location. The volume superiority of EH was due to its greater height, larger DBH, more cylindrical form, and slightly thinner bark. The relative taxa rankings for tree volume in the wood study sample were virtually the same as rankings based on all surviving trees at 8.5-9.5 years of age (e. g., Nikles et al. 1987), suggesting that the wood sample trees were representative of the range of tree sizes.

Table 2. Individual tree volume inside bark and whole core extracted wood density for taxa and locations in Studies 364 and 464.

Taxon	m^3	kg/m^3	m^3	kg/m^3	m^3	kg/m^3	m^3	kg/m^3
364 Location:	Whiporie		Toorbul		Coochin		Byfield	
E	.427a	452	.468b	511a	.355ab	530a	-	-
EH	.569a	438	.700a	475b	.469a	508ab	.523a	480a
H	-	-	.512b	461b	.327b	487b	.472a	475a
464 Location:	Whiporie		Husseys		Tuan		Byfield	
E	.158a	422	.295c	437a	.320b	465a	.166b	482a
BC	.177a	423	.368b	430ab	.300b	468a	.253a	488a
EH_{Be}	.230a	405	.437a	422b	.368a	436b	.304a	464b
EH_{By}	-	-	.400ab	426ab	-	-	-	-
H_{Be}	-	-	.300c	427ab	.391a	431b	.298a	460b
H_{By}	-	-	.383b	420b	-	-	-	-

Age-Age Relationships

Young wood properties in core samples were often correlated with older wood properties in EH cores on both individual and family bases. For example, extracted wood density of rings 0-5 and later segments tended to be correlated with older segments as well as with pith to 10-, 15-, and 19-ring cores in Study 364 and 10-, and 13-ring cores in Study 464 (Table 3). These trends were stronger than in other taxa. Such relationships suggest that EH could be evaluated for wood properties using rings 0-5. Whole core values were used for subsequent analyses.

Location Effects

Extracted wood density tended to increase with decreasing latitude in all taxa (Table 2). Wood density was typically highest at Byfield, the most

Table 3. Age-age phenotypic correlations for extracted core wood density of individual EH trees (I) and EH families (F) by Study 364 locations Toorbul, Coochin, and Byfield, and Study 464 locations Husseys, Tuan, and Byfield.

	Growth Ring Age (years)					
	6-10	11-15	16-19	0-10	0-15	0-19
Age: Loc. Study 364	I / F	I / F	I / F	I / F	I / F	I / F
0-5:To	.68*/.41	.54*/.14	.66*/.41	.89*/.64	.82*/.39	.79*/.21
Co	.26 /.54	-.01 /.53	-.03 /.89	.72*/.83	.53*/.78	.45+/.82
By	.66*/.98+	.45+/.53	.37 /.34	.84*/.99*	.73*/.98+	.70*/.91
6-10:To		.69*/.40	.81*/.57	.92*/.84	.94*/.94	.93*/.96+
Co		.67*/.71	.39 /.87	.82*/.91	.87*/.93	.82*/.91
By		.77*/.67	.56*/.52	.94*/.99*	.93*/.99*	.90*/.98+
11-15:To			.89*/.95+	.62*/-.07	.80*/.69	.75*/.56
Co			.61*/.69	.42+/.69	.70*/.81	.70*/.62
By			.79*/.91	.71*/.60	.81*/.65	.82*/.76
16-19:To				.76*/.19	.88*/.81	.87*/.66
Co				.20 /.99*	.36 /.97+	.55*/.99+
By				.54*/.41	.62*/.51	.73*/.69

	6-10	11-13		0-10	0-13	
Study 464						
0-5:Hu	.68*/.68+	.54*/.52		.87*/.93*	.77*/.87*	
Tu	.45+/.88	.42+/.51		.74*/.90	.74*/.98*	
By	.52*/.59	.30 /-.01		.76*/.89	.71*/.86	
6-10:Hu		.76*/.89*		.91*/.90*	.91*/.94*	
Tu		.54*/.09		.92*/.99*	.88*/.84	
By		.51+/.62		.92*/.89	.90*/.90	
11-13:Hu				.68*/.74+	.73*/.80*	
Tu				.51+/.11	.72*/.62	
By				.48+/.36	.67*/.45	

+ and * - Significant at the 5- and 1-% levels, respectively

northerly location (22°50'S), and lowest at Whiporie, the southernmost location (29°15'S), with a differential of about 10% in Study 364 and some 15% in Study 464. However, in Study 364-Coochin, a very poor site, extracted wood density was higher than at any other Study 364 location. Extractives content also increased with decreasing latitude in the older trees of Study 364, but no such trend was evident in the more recently planted Study 464.

Taxa by Location Interactions

No taxa x location interactions were detected for any wood properties in Study 464 (Table 4). In Study 364, though, extractives content, moisture content, and unextracted wood density displayed interactions due to a change of taxa positions at the Coochin location.

Table 4. Analyses of variance across locations and taxa in Studies 364 and 464 for whole core extractives content, moisture content, extracted wood density, and unextracted wood density.

Source	Extractives Content	Moisture Content	Extracted Wood density	Unextracted Wood density
Study 364:				
Location	NS	NS	*	NS
Taxa	NS	NS	**	NS
Families/T	NS	NS	NS	NS
L x T	**	**	NS	**
L x F/T	NS	NS	NS	NS
Study 464:				
Locations	NS	**	**	**
Taxa	NS	NS	*	*
L x T	NS	NS	NS	NS
Families	*	NS	NS	NS
L x F	NS	NS	NS	NS
T x F	NS	NS	NS	NS
L x T x F	NS	NS	NS	NS

NS, *, and ** - Nonsignificant and significant at the 5- and 1-% levels, respectively

E, H, and EH comparisons

Studies 364 and 464 establish that all taxa have virtually the same extracted wood density in the first five rings, EH achieves a density slightly above that of the H parent as early as 10 rings, and EH has a nearly intermediate density between the parental taxa by 19 rings (Table 2). Extractives content of EH was much less predictable. Latewood percent of EH was typically intermediate between the parental taxa, as was heartwood percent.

Combining Ability of E Parents

General o-p combining abilities of the 10 E parents in Study 464-Husseys were of varying reliability in predicting EH wood properties. For extracted wood density, combining abilities of pure E, EH_{By}, and backcrosses with EH_{Be} were strongly correlated but not with EH_{Be} (Table 5). Thus, the potential for selecting E parents for EH_{By} or backcross breeding based on the densities of their o-p families in traditional breeding programs appears good. In fact, the range of variability in extracted wood density in these breeds is such that EH_{By} and BC families can be generated that equal or exceed the desirably high average wood density of E.

O-p combining abilities of E parents for extractives content were of little use in predicting EH extractives content, another important trait. As for EH_{By} unextracted wood density, though, o-p combining abilities may be helpful in making desirable hybrids with the H_{By} population. Certain E parents had a strong influence on EH extractives content.

Table 5. Correlations among combining abilities of E females in 464-Husseys for 0-13 extracted wood density and 0-5 extractives content of O-P, BC, and EH families.

Combining Ability of E Females in	Combining Ability of E Females in		
	BC Families	EH_{Be} Families	EH_{By} Families
	----------Extracted Wood Density----------		
O-P Families	.67*	.62	.87**
BC Families		.58	.62
EH_{Be} Families			.32
	----------Extractives Content-----------		
O-P Families	.44	.33	.81**
BC Families		-.21	.41
EH_{Be} Families			.06

EH Backcross to E

The BC was often intermediate between E and EH_{Be} in Study 464. In terms of volume inside bark, BC was better than E except at Tuan. Its wood properties frequently were more desirable than those of EH_{Be} except that its extractives content tended to be higher. Accordingly, the BC appears to have potential for Florida and the southeastern United States for a higher growth rate than E with wood properties similar to E; the frost-hardiness and fusiform rust susceptibility of the BC and even EH remain critical traits however.

Hybrid Breeding Strategy

Procedures for the production of EH in Queensland include parent selection, hybrid testing, monoclonal orchards, and vegetative propagation. E parents with good combining ability for volume in o-p families are generally better as EH seed parents, but o-p combining abilities of H parents typically provide little indication of their potential for superior EH families. Consequently, an extensive field testing program is conducted to identify superior EH. Selected E parents are then grafted onto H rootstock to establish monoclonal orchards in the Byfield area that are hedged regularly beginning in the first year to promote early and abundant flower production (Nikles and Robinson 1989). Pollen collected in advance of E flowering from the complementary H parent is mass applied by wanding to produce the maximum possible seed. Due to the large quantity of propagules required, a substantial number of the EH seedlings produced are also hedged to provide commercial rooted cuttings.

CONCLUSIONS

Critical wood properties and bark thickness of EH, associated E and H parents, and BC were assessed. EH wood properties may be evaluated by the first five rings. Density and latewood tended to be greater at poorer sites and also increased with proximity to the equator. Taxa comparisons for density and latewood were E > BC > EH > H; extractives content, EH = H ≥ BC ≥ E; and heartwood percent, minor differences. Extractives in EH and H heartwood exceeded that of E. Large family differences within taxa typically surpassed taxa differences. Location interactions with taxa and families were not observed in the study with 10 families per taxon, but location by taxa interactions were

detected in the study with only four families per taxon. Superior growth and generally acceptable wood properties of EH favor its use over the pure species in appropriate areas of the subtropics. Parental selection, followed by EH evaluation, will insure wood properties comparable to E. Seed production systems and vegetative propagation practices such as those developed at the QFS can be implemented to produce EH if evaluations underway in the subtropics, including recent trials in Florida, demonstrate growth superiority of the EH. Due to its potentially greater frost-hardiness, the BC may be of value in the coastal southeastern United States.

LITERATURE CITED

Bragg,C.T. 1990. Machine stress grade and resinosis comparisons: slash pine, Caribbean pine, and hybrids. QFS Forest Development Branch F_1 Hybrid Review Seminar, Gympie, 9-10/10/90.

Harding,K.J., and D.B.Eccles. 1990. Studies of wood properties in 13-year-old slash x Honduras Caribbean pine F_1 hybrids and their parental species. QFS Forest Development Branch F_1 Hybrid Review Seminar, Gympie, 9-10/10/90.

Harding,K.J., and M.T.Hagan. 1990. Variation in wood properties of 24.5-year-old slash x Honduras Caribbean pine F_1 hybrid stems compared to their parental species properties when grown on a well drained site. QFS Forest Development Branch F_1 Hybrid Review Seminar, Gympie, 9-10/10/90.

Last,I.S. 1990. Status of the PEE x PCH F_1 hybrid in Queensland forestry and strategies for further development. QFS Forest Development Branch F_1 Hybrid Review Seminar, Gympie, 9-10/10/90.

Nikles,D.G. 1991. Increasing the value of future plantations in Argentina and southern Brazil using slash x caribbean pine hybrids developed in Queensland. Jornada sobre Pinus caribaea, Eldorado, Argentina, April 25-26, 1991.

Nikles,D.G., P.C.Bowyer, and R.L.Eisemann. 1987. Performance and potential of hybrids of slash and Honduras Caribbean pines in the subtropics. P.68-79 in Proc. CIEF Simposio sobre Silvicultura y Mejoramiento Genetico de Especies Forestales, Buenos Aires, Vol. 1.

Nikles,D.G., and M.Robinson. 1989. The development of Pinus hybrids for operational use in Queensland. P.272-282 in Breeding Tropical Trees: Population Structure and Genetic Improvement Strategies in Clonal and Seedling Forestry.

Smith,W.J. 1977. Variation in wood quality and productivity of some Queensland plantation-grown softwoods. P.74-99 in Proc. Joint Workshop Progress and Problems of Genetic Improvement of Tropical Forest Trees, Vol. 1.

Vanclay,J.K., and P.J.Shepherd. 1983. Compendium of volume equations for plantation species used by the Queensland Department of Forestry. Queensland Department of Forestry Technical Paper No. 36.

CLONAL SELECTION AND TESTING OF VIRGINIA PINE FOR CHRISTMAS TREE CHARACTERISTICS

G.F. Brown[1] and G.S. Foster[2]

Abstract. -- Two and one-half year-old Virginia pine (Pinus virginiana Mill.) trees were selected for superior Christmas tree characteristics from a large Christmas tree grower's plantation. Three-inch-long stem cuttings were collected from the select trees and rooted using Hare's powder and a rooting bench. Rooted cuttings from 47 clones were field planted in spring 1987 near Hattiesburg, Mississippi and subsequently were cultured with standard Christmas tree practices, including shearing. The sole cultural treatment to be tested was the effect of staking versus not staking each clone.

Following four seasons of growth and culture, the trees were assessed for total height and overall quality rating. The unstaked ramets of each clone were as straight as the staked ramets. Highly significant clonal variance was apparent for total height with an average of 129.21 cm and a range of clone means of 102.44 to 160.00 cm. The top ten percent of the clones averaged 21.16 cm taller than the population average. Broad-sense heritability for height on a individual basis was .21. Based on a five-point scale, there were highly significant differences among clones in quality rating. The top ten percent had an average rating of 4.57 compared to the overall mean of 3.64. The broad sense heritability for the rating was .31.

Additional keywords: Pinus virginiana, vegetative propagation, rooting success, heritabilities, staking

INTRODUCTION

Selection and clonal propagation of superior trees are well established procedures in horticulture and in reforestation for a few tree species. The Christmas tree industry has made a few attempts to develop superior clones for their use (e.g., Proebsting 1981), but to date none of these efforts seems to have succeeded in an operational sense. Given that Christmas tree production and sales represent an important business in the U.S., development of large-scale clonal selection and propagation procedures will enhance the success of the industry.

Little work has been done to improve most Christmas tree species genetically, with the most advanced program being with Virginia pine (Pinus virginiana Mill.). In this case, a seed orchard based tree improvement program is expected to produce genetically superior seedlings for the Christmas tree industry (Brown 1987). Development of cloning procedures applicable to large-scale use has been limited to a single published report by Snow and May (1962). Their best treatment for rooting of stem cuttings yielded an average of 72 percent rooting, hence the species appears to be at least moderately amenable to the rooted cutting process.

With the large potential impact of such a program on the Christmas tree industry and the paucity of clonal production information in mind, a pilot scale study was initiated with Virginia pine. The objectives were to: (1) assess the growth and form of Virginia pine rooted cuttings in an operational Christmas tree production system, (2) determine whether initial staking of the rooted cuttings influenced subsequent straightness of the resultant trees, and (3) measure the degree of genetic control of height growth and quality rating among Virginia pine clones.

MATERIALS AND METHODS

Phenotypically superior trees were selected from cultured plantations managed by Sunrise Tree Plantation Co. located near Hattiesburg, Mississippi. The trees were 2-1/2 or 3-1/2 years old from seed, and they were planted as 1-0 bareroot seedlings. The seed for the seedlings arose from bulk lots of seed from the Kimberly Clark Co. or the Bowaters Co. first generation seed orchards. The 47 select trees were chosen for superior height, straightness, a preponderance of four or more branches in each branch whorl, and good overall Christmas tree quality.

Stem cuttings were collected on July 8, 1986 for subsequent propagation. Following collection, the cuttings were placed in plastic bags, sealed, and put in an ice chest. The cuttings were set the next day for rooting at International Forest Seed Co., Odenville, Alabama. Their standard rooting procedures (Hughes 1987; Foster 1990) were used, and cutting length was standardized to three inches. The cuttings were established in 5.5 cu inch plastic Hiko containers in a greenhouse rooting area in a randomized complete block design with four replications of five cutting plots. The rooting process required about four months in the greenhouse, and an additional five months were needed to first harden-off the trees and then initiate additional new growth prior to outplanting.

The trees were planted on April 21, 1987 at Sunrise Tree Plantation. A completely randomized design was used with 3 to 5 rooted cuttings (mainly 4) for each of 47 clones. Single tree plots were used with a 6 x 6 ft spacing. Half of the ramets of each clone were staked to encourage erect growth. The experiment was located in the middle of an operational Christmas tree plantation. Test trees were cultured normally including shearing, grass and weed control, and insecticide application.

The test was measured in the fall, 1990 following four growing seasons, and the data were analyzed. A least squares analysis of variance was used (PROC GLM, type III sums of squares; SAS 1985), and the coefficients of variance components were adjusted for data imbalance (Hartley 1967; Goodnight and Speed 1978). The form of the analysis of variance is given in Table 1. Mean squares were equated with sums of squares, and variance components were derived (Kempthorne 1969). Variance components were also expressed as the percentage of the total variance accounted for by each source of variation. Staking treatments were fixed effects, and clones were random effects. Broad-sense heritabilities were calculated on an individual-ramet basis (H^2) and also on a clone-mean basis ($H_{\bar{x}}^2$).

$$H^2 = \frac{\sigma^2_c}{\sigma^2_c + \sigma^2_{sc} + \sigma^2_e} \qquad\qquad H_{\bar{x}}^2 = \frac{\sigma^2_c}{\sigma_c + \dfrac{\sigma^2_{sc}}{s} + \dfrac{\sigma^2_e}{ns}}$$

where,

σ^2_c = variance among clones

σ^2_{sc} = variance due to interaction of staking and clones

σ^2_e = error variance

s = number of staking treatments

n = number of ramets per clone

The later heritability is appropriate in reference to selection based on clone-mean performance. It should be noted that the magnitude of the clonal variance component and also heritability are inflated by the size of the clone x location interaction since the test was located at a single site. Expected genetic gain can be predicted by multiplying broad-sense heritability based on clone means by the selection differential. Theoretically this value will apply only to the same site type as where this genetic test was growing.

RESULTS AND DISCUSSION

The ANOVA for both height and the quality rating is given in Table 2. The only significant source of variation was the effect of clones, which also accounted for a relatively high percent of the total variance. It is interesting to note that the effect of staking at time of planting was not significant for either of the measured characteristics nor did it account for much of the total variance.

243

Table 1. Form of the analysis of variance for four-year height and quality rating of Virginia pine clones.

Source of variation	Degrees of freedom	Expected mean squares
Stake Tmnt (s)	1	$\sigma^2_e + 1.634\,\sigma^2_{sc} + 66.831\,\Theta^2_s$
Clones (c)	46	$\sigma^2_e + 3.157\,\sigma^2_c$
S * C	42	$\sigma^2_e + 1.634\,\sigma^2_{sc}$
Error	68	σ^2_e
Total	157	

Table 2. Analysis of variance for height and quality rating of Virginia pine clones.

Source of variation	Degrees of freedom	Height Mean square	Variance component	% of total variance	Quality Rating Mean square	Variance component	% of total variance
Stake Tmnt (s)	1	721.54NS	6.24	1.79	0.4184NS	.0005	0.08
Clones (c)	46	444.27**	71.73	20.55	0.9881**	.1845	31.24
S * C	42	304.79NS	53.23	15.25	0.3826NS	< 0	0.00
Error	68	217.81	217.81	62.41	0.4055	.4055	68.67
Total	157						

** Significant at p<0.01.
NS Non-significant at p>0.05.

After four years, 158 out of the original 184 rooted cuttings (85.9% survival) were still alive. Twenty-five of the clones had not lost any ramets, while 18 clones had lost 1 ramet and only 4 clones had lost 2 ramets. Even though there is insufficient data to test effectively, there do not appear to be any clonal effects on survival.

The mean height for all trees was 129.21 cm (Table 3) which is considerably less than previously reported heights for three-year-old trees (Brown 1987). The trees in this test had been sheared twice a year beginning in their third year, which would reduce their total height. Also, the rooted cuttings were planted fairly late (April) and the normal amount of growth usually observed on seedlings was not obtained during

the first season. By the second year, the trees appeared to grow more normally. By the fourth year, the trees appeared to be very normal, except their height was comparable to sheared three-year-old trees.

Table 3. Clone means, ranges, heritabilities and select clone means.

Variable	---------------------- All 47 Clones ----------------------					Best 5 Clones Means
	Mean	Max	Min	H2	H$\frac{2}{x}$	
Height (cm)	129.21	160.00	102.44	0.21	0.43	150.37
Quality rating (score)	3.64	4.67	2.33	0.31	0.59	4.57

Progeny tests previously evaluated by the author all had mean quality ratings between 3.0 and 3.4. The quality rating of 3.64 observed on this test indicates that the quality of the trees is at least as good as trees grown from seedlings, if not a little better. A higher quality rating should be expected because of the select nature of the clones. The overall improved quality was also commented on by the cooperator at Sunrise Tree Plantation who stated that the quality was better than normal, but that the growth was less by approximately one year. It appears that we can achieve quality Christmas trees from rooted cuttings, but that their initial growth may be delayed, especially if planted late.

It is not clear why the staking treatment had no effect on tree quality. Normally, a crooked stem is the single largest factor affecting quality. In this test, most of the trees appeared to be growing straight with very few leaning to one side. There is the possibility that because the first-year growth was less than normal, the root systems had more opportunity to develop prior to the top growth, creating more wind firm trees. Another possible explanation could be that all of the original select trees were picked because of their straightness. Both of these factors are confounded and may explain the lack of effect of staking.

Previously reported narrow-sense heritabilities for height based on half-sib progeny tests of Virginia pine were 0.19 for first generation tests (Brown 1987), 0.39 for unsheared second-generation trees and 0.20 for sheared second-generation trees (Brown 1990). It appears shearing lowers the heritability estimates by introducing a new source of environmental variance. The broad-sense heritability calculated on an individual-ramet basis (0.21) in this test agrees very closely with previous results. Broad-sense heritability is theoretically larger than narrow-sense heritability due to inclusion of non-additive genetic variance in the numerator. Although the narrow-sense heritability estimates arise from a different study (lessening their comparability), the closeness of size indicates a preponderance of additive genetic control of height growth. It appears that the heritability for height growth of sheared Virginia pine

Christmas trees will be approximately 0.20, which is sufficient to allow for selection and improvement in a breeding program.

The quality rating heritability of 0.31 calculated in this test is considerably higher than previous results of 0.16 for first-generation tests, 0.21 for unsheared second-generation trees and 0.13 for sheared second-generation trees (Brown 1990). The quality of the clones is more consistent within a clone than the quality within half-sib families. The clonal source of variation accounted for almost 50 percent of the total variance in quality rating, which is high. With similar rationale as above, quality rating may be under both additive and non-additive genetic control, resulting in higher broad-sense heritabilities. This may be an indication that the use of vegetatively propagated clones may result in higher gains in quality rating than the use of improved seed from a breeding program.

The analysis of both height and quality rating indicates that rooted cuttings can be used for the production of Christmas trees. The means of the best five clones (top ten percent) combining both quality and height (Table 3) indicate that the use of these clones would result in a quality improvement of 0.93 in rating and 21.16 cm in height compared to the overall means of the test. However, it should be noted that no seedling check nor other non-select clones were planted for comparison purposes, therefore, realized gains could not be calculated. Both of these gains are greater than those previously estimated from a breeding program (Brown 1987).

CONCLUSIONS

Virginia pine rooted cuttings appear to grow normally, especially when fairly juvenile ortets are used. The overall quality rating of the trees is as good and possibly better than that experienced from half-sib progeny tests. The overall height growth was less. The trees after four years in the field were very similar in height to three-year-old trees from seedlings. Additional studies will be needed to compare rooted cuttings and seedlings to assess the normalcy of height growth. There was no effect of staking observed in this test and it may not be of value as a general practice. This is not meant to imply that staking a leaning tree is of no value, but rather the random staking of newly planted rooted cuttings showed no effect.

Based on the relatively high estimates of heritabilities and their agreement with previous estimates, the height and quality of Virginia pine under similar cultural practices appear to be under moderate genetic control. The cultural practices required to produce quality Christmas trees are intensive and were thought by some to totally overwhelm the genetic component. Based on this research, the genetic component is still important, even when subjected to intensive cultural practices. Additionally, the practicality of clonal selection of Virginia pine for Christmas trees seems good.

LITERATURE CITED

Brown, G. 1987. Genetic improvement of Virginia pine Christmas trees. Proc. 19th South. For. Tree Imp. Conf., College Station, TX. pp.9-16.

Brown, G. 1990. Heritabilities of sheared Virginia pine Christmas trees. School of Agriculture Annual Research Report, Alabama A&M Univ. 1990. In Press.

Foster, G.S. 1990. Genetic control of rooting ability of stem cuttings from loblolly pine. Can. J. For. Res. 20:1361-1368.

Goodnight, J.H., and F.M. Speed. 1978. Computing expected mean squares. SAS Institute Inc., Cary, NC. Tech. Rep. R-102.

Hartley, H.O. 1967. Expectation, variances and covariances of ANOVA mean squares by "synthesis". Biometrics, 23:105-114.

Hughes, H.F. 1987. Cutting propagation of rust resistant hedges of Pinus taeda. Plant Propag. 1:4-6.

Kempthorne, O. 1969. An introduction to genetic statistics. Iowa State University Press, Ames.

Proebsting, B. 1981. Every Christmas tree a '10'. Orn. Northwest 5:14-15.

SAS Institute Inc. 1985. SAS user's guide: statistics, version 5. SAS Institute Inc., Cary, NC.

Snow, Jr., A.G., and C. May. 1962. Rooting of Virginia pine cuttings. J. For. 60:257-258.

IMPACT OF NURSERY MANAGEMENT PRACTICES ON HERITABILITY ESTIMATES AND FREQUENCY DISTRIBUTIONS OF FIRST-ORDER LATERAL ROOTS OF LOBLOLLY PINE

P.P. Kormanik[1], H.D. Muse[2] and S.J. Sung[1]

Abstract.--Frequency distribution and heritability of first-order lateral root (FOLR) numbers in 1-0 seedlings were followed for 5 years for 115 different half-sib seedlots from the Georgia Forestry Commission's Arrowhead and Baldwin Seed Orchards. In 1986 and 1987, seedlings were permitted unrestricted growth under management conditions similar to those practiced in most forest tree nurseries in the Southern United States. Seedlings were placed in four different classes based on the number of FOLR with proximal diameters above a threshold value. These classes were 0-3, 4-5, 6-7, and ≥ 8 FOLR. In 1988, 1989, and 1990, management practices were altered to restrict height growth by 30 to 40 percent for each of four FOLR groupings. The associated reduction in seedling sizes had very little affect on either FOLR family mean heritability estimates or the assumption of truncated normality for FOLR data. Restricting growth did reduce FOLR proximal diameters, but in each data set the seedlings in the FOLR group with the fewest FOLR (0-3 in 1986 and 87, 0-2 in subsequent years) were always significantly smaller than those in the other groups. Not only were seedlings in this first FOLR group of smaller size, they also had unfavorable traits: succulent stems, preponderance of primary needles, and poorly developed or absent terminal buds.

Key Words: restricted and unrestricted growth, artificial regeneration

INTRODUCTION

Protocols for predicting the growth potential of progeny from specific mother-trees have been under development since tree improvement programs were initiated in the late 1940s. Heritability trials for tracking specific traits are standard procedures in all tree improvement programs. However, heritability estimates for given morphological and physiological traits vary significantly among different tests, and between fertility levels in the same tests, and estimates for juvenile and mature trees are often poorly correlated (Zobel and Talbert 1984, Bailian et al 1989, Gerhold and Stanton 1987, Bridgwater and Williams 1987). It is difficult to identify the causes of variation in heritability estimates for a specific trait because heritability estimates carry

---

[1] Institute of Tree Root Biology, Forestry Sciences Laboratory, Athens, Georgia and [2] Department of Mathematics and Computer Science, University of North Alabama, Florence, Alabama.

large associated errors, even when large numbers of families and progeny are used (Zobel and Talbert 1984). Silviculturists and forest managers generally agree that early testing for traits that influence progeny performance and subsequently improved yields are necessary if we want meaningful gains in the near future.

Before inorganic fertilizers, pesticides, and modern irrigation systems were heavily used in forest tree nurseries, seedling grading systems based primarily upon stem morphological characteristics proved effective (Wakeley 1954). Growing conditions were not ideal, seedling growth was unrestricted, and the grading systems identified small noncompetitive seedlings. Modern management practices, however, significantly increased the sizes of all the southern pine seedlings in seedbeds. As a result, morphological grading no longer worked well, and it rapidly fell from favor among nursery managers.

In both tree improvement programs and artificial regeneration systems in the Southern United States, the production of seedlings of uniform size became highly desirable. Seedling production methods, therefore, were altered to restrict the growth of the fastest growing seedlings (USDA 1985). Greater uniformity in seedling sizes was generally achieved by restricting growth with mechanical procedures such as top clipping, root wrenching and undercutting. Uniformity had a considerable biological cost. The more vigorous and competitive seedlings were adversely affected by repeated top clipping while the slower growing noncompetitive seedlings benefited. These mechanical procedures produced uniform seedlings in which differences in growth potential after outplanting could not be identified.

In recent studies of the heritability and frequency distribution of numbers of first-order lateral roots (FOLR) in half-sib loblolly pine progeny, the seedlings were grown using currently accepted fertility and water regimes in southern forest tree nurseries but growth of seedlings was unrestricted (Kormanik and Muse 1989, Kormanik et al. 1990). In these early trials, even the smallest seedlings in practically all of the half-sib seedlots were from 12 to 20 cm taller than Wakeley's grade 2 seedlings and had comparable or larger root collar diameters (RCD) (3.2 mm). Heritability estimates of FOLR numbers, based on plot means, were found to be ca 0.76 $\pm$ .09 (Kormanik et al. 1990). Over 13,000 seedlings were established over a 2-year period in field research studies. Only diseased and mechanically damaged seedlings were excluded from these outplanting trials. Even though severe droughts occurred throughout the Southeast during this period, survival of these seedlings averaged between 80 and 85 percent.

Identification of FOLR characteristics was based upon unrestricted growth of seedlings that permitted nursery bed competition ($280/m^2$) but resulted in favorable root/top ratios. Such large seedlings were undesirable for out-planting in a commercial operation. Even the progeny of the best mother trees were not uniform in size. Unrestricted growth, under these conditions, allowed the competitive ability of seedlings in the seedbeds to be expressed. Non-competitive individuals all appeared to share several undesirable stem traits such as succulent stems, little secondary needle development, and lack of terminal buds. These undesirable traits were also noted by Wakeley (1954), but have received little emphasis since the late 1950s because mechanical procedures have been used to obtain seedling uniformity since that time.

Since changes in management practices significantly altered the value of stem morphology as an indicator of quality (Wakeley 1954), we became concerned that management practices that restricted seedling development might also affect the lateral root morphology. If so, heritability estimates and our ability to assess seedling competitiveness based upon root morphological development might also be affected. These concerns led to the studies we now describe.

METHODS

All study seedlings were grown at the Institute of Tree Root Biology (ITRB), Whitehall Experimental Nursery, Athens, Georgia. All mother-tree seedlots were obtained from the Georgia Forestry Commission, which collected them in 1986 through 1990.

Procedures for growing seedlings with unrestricted growth in 1986 and 1987 are reported in detail elsewhere (Kormanik et al. 1990). Progeny from 12 mother trees in 1986 and 25 mother trees in 1987 were included in the test. The two criteria of FOLR were: (1) regardless of length, diameter at the proximal end ≥ 1 mm; and (2) maturity, rigidity, and suberization of the periderm.

In 1988, 34 half-sib seedlots were used. Total nitrogen application was reduced from approximately 70 ppm to 25 ppm (rates equivalent to 140 and 50 lbs N/acre) for the entire growing season. Nitrogen (NH_4NO_3) was applied at 2.5 ppm (5 lbs N/acre) in mid-May and again in the last week of May; and at 5 ppm (10 lbs N/acre) on June 5, June 23, July 2, and July 20. Fall N application was avoided to enhance fall foliar discoloration common to loblolly pine seedlings. The proximal diameter of roots to be counted was reduced to 0.5 mm to reflect the reduction in seedling sizes resulting from reduced soil fertility, and the FOLR count classes were altered to 0-2, 3-5, 6-7 and ≥ 8 to reflect a reduction in overall seedling development. The FOLR classes used in 1986-1987 were 0-3, 4-5, 6-7, and ≥ 8.

Water was applied as needed until the final nitrogen top dressing. After that, water was applied weekly at 3.0 cm per week when rainfall did not occur or was limiting. Watering was continued until first frost in mid-November. The seedlings were lifted and outplanted in late January. A total of 200 seedlings from each mother tree (100 from each replication) were included in the out-planting, and only mechanically damaged or rust-infected seedlings were excluded from the data set. No more than 1 percent of the seedlings in any family were infected with rust. Approximately 6,800 graded seedlings grown in the 1988 nursery trial were outplanted.

In 1988, we began to assay seedlings for the enzymes involved in sucrose utilization to assess sink strengths of roots and tops throughout the year. The three enzymes that catalyze sucrose breakdown (sucrose synthase, acid invertase, and neutral invertase) were assayed according to procedures reported by Sung and others (1989a and b). The purpose of these assays was to determine if seasonal patterns in the activity of these three enzymes could help in clarifying the causes for the periodicity in growth between roots and tops of loblolly pine seedlings. Beginning in mid-July and continuing for 12 months, the activities of sucrose breakdown enzymes in the taproot and stem cambial tissue extracts were measured biweekly with a Beckman DU-7 spectrophotometer. Composite samples

of 3 g of cambial tissue were obtained by scraping the exposed cambial surface
of 15 to 50 debarked stems or roots. More seedlings were needed earlier in the
season and fewer later as the seedling sizes increased. Activities of all three
sucrose breakdown enzymes were assayed from a single root or stem extract.

In 1989, 34 loblolly pine half-sib seedlots were included in the test.
Essentially the same management protocol as in 1988 was followed throughout the
growing season. In mid-September, after budset was completed in over 90 percent
of the seedlings in all seedlots, nitrogen was applied at the rate of 10 ppm
(rate equivalent to 20 lbs N/acre) to enhance carbon production. Based on 1988
enzyme assays, we believed that this was when carbon would be shunted to the
roots. The total amount of N applied was 35 ppm (rate equivalent to 70 lbs
N/acre). Again, 100 seedlings from each replication from each mother tree were
lifted in mid-December. None of the seedlings in this trial were outplanted.
However, over 2,000 seedlings were transplanted to other nursery beds and during
the next 12 months sucrose metabolism was monitored in these transplants by
periodically assaying the three enzymes followed in 1988.

During the 1990 growing season, 24 additional half-sib seedlots were
included in the trials. The quantity of nitrogen applied by mid-July was the
same as in the previous 2 years, but at bud set in September only 7.5 ppm (rate
equivalent to 15 lbs N/acre) were applied. The total for the year was thus 30
ppm (rate equivalent to 60 lbs N/acre). Watering was altered from previous
trials. In 1990, three model R irrometers (Irrometer Company, Riverside, CA)
were placed in each bed; all were placed in the approximate bed center to a
depth of 15 to 20 cm (6 to 8 inches). One was placed in the middle of the bed
and the other two were placed approxi- mately 3.5 (ca 10 feet) from either end.
After the last nitrogen treatment in mid-July, supplemental watering was used
only after the irrometers within a bed all registered 70 centibars for 3
consecutive days. At that time, 3 cm of water were applied. The seedlings were
harvested in late February. No field testing was done, but more than 1,000
seedlings were transplanted within the nursery to follow the seedlings for one
additional year. The same number of seedlings from each mother tree were used
in the heritability data set as in previous trials.

ANALYSIS

Data from each experiment were examined by ANOVA. For each year's data, the
FOLR sample cumulative distributions for individual families were compared using
the Smirnov nonparametric procedure (Conover 1980). Underlying theoretical
distributions for FOLR were then examined using Kolmogorov's goodness of fit
procedures. For each year's data and for the combined data, hypotheses of
approximate normality and truncated normality were tested using nontransformed
and square-root transformed FOLR values.

Family mean heritability was estimated for each year's data based on the
analysis of variance of plot means. Calculations were based on h^2 =
(MSF-MSFxR)/MSF where MSF and MSFxR denote the respective mean squares for
family and family by replication interaction. Since the data for many families
did not fit a normal distribution, the within-plot variance estimate was not
utilized in estimating heritability. It is recognized that failing to correct
for within-plot variance tends to inflate the heritability estimate; however,

since individual family sample sizes were large, the resulting inflation was considered negligible.

RESULTS AND DISCUSSION

The ANOVAs used to calculate heritability estimates for the data set for each year are shown in Table 1, and the heritability estimates are shown in Table 2. The percentage of seedlings in each FOLR group and average heights and root collar diameters of the seedlings for all combined mother trees are shown in Table 3.

Table 1. ANOVA of FOLR plot means for loblolly pine for years 1986-1990.

Source	df	Sum of squares	Mean squares	Variance component est.
		1986		
reps	3	7.282634	2.437545	
families	11	30.149740	2.740885	0.527047
fam x reps	33	20.879008	0.632697	0.632697
		1987		
reps	1	0.083988	0.083988	
families	24	22.516832	0.938201	0.359221
fam x reps	24	5.274240	0.219760	0.219760
		1988		
reps	1	0.334448	0.334448	
families	33	20.402580	0.618260	0.197262
fam x reps	33	7.350273	0.222736	0.222736
		1989		
reps	1	0.441934	0.441934	
families	19	8.005344	0.421334	0.158946
fam x reps	19	1.965398	0.103442	0.103442
		1990		
reps	1	2.618002	2.618002	
families	23	13.903398	0.604496	0.209351
fam x reps	23	4.273248	0.185793	0.185793

In 1986 and 1987, seedlings were permitted unrestricted growth under soil fertility and water regimes comparable to those in most southern pine nurseries (Kormanik et al. 1990, USDA 1985). The one exception was that we used less nitrogen top dressing and eliminated fall applications of both nitrogen and potash. Seedling sizes, heritability estimates, and FOLR groupings were comparable for these 2 years (Table 2 and 3). The initial field survival ($\geq$80%) and first year development of these large seedlings, both established during the

Table 2. Family mean heritability estimates for number of FOLR of loblolly pine seedlings.

Year	No. families	h^2 est	S.E.
1986	12	0.769	0.170
1987	25	0.766	0.292
1988	34	0.640	0.140
1989	20	0.754	0.138
1990	23	0.693	0.158
Pooled*		0.712	0.088

* Pooled data is presented only to illustrate the uniformity of the data sets in spite of the altered nursery practices and were not used in statistical analyses reported here.

extreme drought years of 1987 and 1988, were satisfactory. It was obvious, however, that the seedlings were large enough to cause problems for commercial machine planting and too large for typical hand planting.

Stem heights and RCD were smaller in all FOLR groups in 1988 than in 1986-87, when growth was restricted (Table 3). Normal fall needle discoloration occurred but no attempt was made to eliminate it through fall application of nitrogen. First-year survival of these seedlings was ca 85 percent at the Savannah River Forest Station, Aiken, South Carolina.

In 1989, the nitrogen applied in September prevented normal needle discoloration during the winter but did not result in any obvious elongation in stems and little differences in FOLR proximal root diameters. The heritability values were numerically comparable to those in the 1986-87 tests and not significantly different among the five data sets (all differences are less than 1 standard deviation). A beneficial response to the September nitrogen application was more abundant feeder root and mycorrhizal development that seemed to accompany the increase in fall root metabolism. We did not have a nitrogen control to verify this observation, but increased mycorrhizal development after mid-August has been reported by others (Marx et al. 1986, Marx and Cordell 1987).

In 1990, we again topdressed seedlings with nitrogen in the fall but we also began controlling moisture after the final nitrogen application in mid-July. Monitoring moisture allowed us to more closely control height growth. This further reduction in top growth did not affect the heritability estimates of FOLR development and yet resulted in desirable RCDs. The reduction in percentage of seedlings in the lowest FOLR group here was due directly to six families. In each of the previous 5 years, different half-sib seedlots were used and normally only one to three lots produced seedlings with significantly lower percentages of seedlings in the lowest FOLR group. In 1990, 6 of the 24 seedlots had 11 percent or fewer seedlings in the lowest FOLR group. This difference, however, did not significantly affect the heritability estimates or

Table 3. Average heights and root collar diameters by FOLR groups for 1986-1990 studies for combined families[1]

FOLR group	Total seedlings (%)	Height (cm)	RCD (mm)
	1986		
0-3	36	40.4	4.0
4-5	25	46.4	5.1
6-7	21	48.2	5.8
>8	18	49.8	6.5
	1987		
0-3	38	38.4	3.8
4-5	20	46.5	5.4
6-7	18	49.1	6.0
>8	24	50.0	7.1
	1988		
0-2	33	28.9	3.2
3-5	47	32.8	4.0
6-7	15	34.4	4.6
>8	5	35.0	5.0
	1989		
0-2	35	27.4	2.8
3-5	36	32.9	4.0
6-7	16	34.7	4.7
>8	13	36.4	5.5
	1990		
0-2	18	22.7	3.2
3-5	49	26.5	4.2
6-7	20	29.1	4.8
>8	13	29.5	5.4

[1] Compiled data sets on file for each mother tree by Georgia Forestry Seed Orchard designation at the Institute of Tree Root Biology, Athens, Georgia.

the truncated normality of this data set compared to those for other years. As in 1989, fall application of nitrogen appeared to enhance root and mycorrhizal development in comparison with development in years when no nitrogen was applied in the fall.

When it became apparent in the mid-1980s that FOLR numbers were associated with competitive ability of open-pollinated seedlings in the nursery (Kormanik 1986), studies were initiated at the Institute of Tree Root Biology to determine the approximate heritability of FOLR numbers for loblolly pine. During the past

5 years, it has become clear that half-sib progeny are not equal in their competitive potential even against their own siblings in nursery beds. We also determined that nitrogen and supplemental watering could be used to regulate seedling sizes. These cultural treatments to control seedling growth had only a minor influence on root morphology. Silviculturally, the FOLR relationship is of considerable interest because it provides a basis for grading loblolly pine seedlings according to their early competitive potential.

When assessing root development of loblolly pine we had noted that regardless of seedling development in different State nurseries, low numbers of FOLR were associated with specific undesirable stem characteristics. These traits were similar to those reported by Wakeley (1954) for his small cull or grade 3 seedlings. While these stem characteristics were useful as indicators when we set up the FOLR groupings used in the initial heritability studies in 1986-87, we had not expected them to be expressed when we permitted unrestricted growth of seedlings (Kormanik and Muse 1986, Kormanik et al. 1990).

In 1988, seedlings were sampled throughout the year to biochemically assess stem and root growth and development. As in sweetgum, pecan, potato, and lima bean (Sung et al. 1989a and b), sucrose synthase activity was the dominant sucrose breakdown enzyme activity in stems and roots of loblolly pine. The seasonal patterns of sucrose synthase activities in stems and in roots coincided with stem and root morphological development reported by Wakeley (1954). Sucrose synthase activity in seedling roots lagged behind that in stems until early fall. Throughout the winter, roots continued to utilize sucrose, but at a slower rate than in the fall. Stem sucrose synthase activity was lowest during the winter and did not return to a high level until mid-March. During active shoot elongation, the previous year's stems and roots were both competing for sucrose with newly formed shoots. In April and May, stem sucrose synthase activity decreased 40% and root activity decreased 70% from levels measured in March. Neutral invertase activities in stems and roots were lower than those of the other enzymes throughout the year, and no seasonal patterns were detected. Acid invertase was more active than neutral invertase at all times. Acid invertase activity sometimes was higher than sucrose synthase activity. We are in the process of correlating acid invertase and sucrose synthase activities in seedlings under various environmental stresses.

High enzyme activity in stems reflects high use of sucrose for stem elongation. Our approach to restricting top growth therefore was to limit the nitrogen supply when stems were elongating. We did not know how these changes would affect FOLR development, but we expected significant changes in heritability estimates based on other published papers. The expected changes did not occur (Table 2). FOLR diameter at the proximal end declined when top growth was reduced, but other morphological characteristics of FOLR did not change. The reduction in FOLR diameter would have resulted in an increase in percentage of seedlings in the smallest FOLR category in 1988 if the 0-3 category had been maintained. This reduction in class ranges had little effect on heritability estimates (Table 2), which are based on total number of FOLR and not on FOLR classes. On the basis of stem characteristics--stem succulence, high proportion of primary needles, and lack of or poor development of terminal buds associated with individual FOLR numbers--the critical FOLR diameter was reduced from 1.0 mm to 0.5 mm.

CONCLUSION

Restricting loblolly pine seedling height growth by taking advantage of carbon allocation distribution patterns does not affect the FOLR heritability estimates or the truncated normality observed in loblolly pine seedlings with unrestricted growth. Morphological stem characteristics common to seedlings in the 0-3 FOLR group with unrestricted top growth were found in the 0-2 FOLR group when growth was restricted. Thus it appears that restricting seedling development in the nursery by reducing soil fertility when stems are elongating will permit identification of noncompetitive seedlings. Such identification has important practical implications. FOLR contributions to later competitiveness are currently being tested in field plantings.

LITERATURE CITED

Bailian, Lai; S.E. McKeand, and H.L. Allen. 1989. Early selection of loblolly pine families based on seedling shoot elongation characters. pp 228-234 in Proc. 20th Southern Tree Improvement Conference, Charleston, SC.

Bridgwater, F.E. and C.G. Williams. 1987. Early testing and juvenile selection in loblolly pine. pp 1-7 in Proc. 30th Northeastern Forest Tree Improvement Conference, July 1986, Orono, ME.

Conover, W.J. 1980. Practical non-parametric Statistics, Ed. 2. Wiley, NY.

Gerhold, H.D. and B.J. Stanton. 1987. Selection efficacy in young black cherry progeny tests. pp 73-81 in Proc. 30th Northeastern Forest Tree Improvement Conference, July 1986, Orono, ME.

Kormanik, P.P. 1986. Lateral root morphology as an expression of sweetgum seedling quality. For. Sci. 32:595-604.

Kormanik, P.P. and H.D. Muse. 1986. Lateral roots a potential indication of nursery seedling quality. pp 187-190 in TAPPI Proc. 1986 Research and Development Conference, Raleigh, NC.

Kormanik, P.P., J.L. Ruehle, and H.D. Muse. 1990. Frequency distribution and heritability of first-order lateral roots in loblolly pine seedlings. For. Sci. 36:802-814.

Marx, D.H. and C.E. Cordell. 1987. Triadimefon affects *Pisolithus* ectomycorrhizal development, fusiform rust, and growth of loblolly and slash pines in nurseries. U.S. Dep. of Agric., For. Serv. Res. Paper SE-267.

Marx, D.H., C.E. Cordell and R.C. France. 1986. Effects of triadimefon on growth and ectomycorrhizal development of loblolly and slash pines in nurseries. Phytopathology 76:824-831.

Sung, S.S., P.P. Kormanik, D.P. Xu, and C.C. Black. 1989. Sucrose metabolic pathways in sweetgum and pecan seedlings. Tree Physiol. 5:39-52.

Sung, S.S., D.P. Xu and C.C. Black. 1989. Identification of actively filling sucrose sinks. Plant Physiol. 89:1117-1121.

USDA Forest Service. 1985. Southern pine nursery handbook. U.S. Dep. of Agric. For. Serv., Southern Region Cooperative Forestry, Atlanta, GA.

Wakeley, P.C. 1954. Planting the southern pines. U.S. Dep. of Agric., For. Serv., Agriculture Monograph No. 18, Washington, DC.

Zobel, B.S. and J.T. Tolbert. 1984. Applied forest tree improvement. Wiley, NY.

GROWTH AND ISOZYME ALLELE FREQUENCY CORRELATIONS IN BLACK WALNUT.

F. H. KUNG, G. RINK, AND G. ZHANG [1]

Abstract.--Average height and diameter of black walnut trees in two progeny test plantations in southern Illinois were measured at age 19. Seed from fast and slow growing families were collected in 1989. Isozymes extraction and analysis was applied to embryos removed from these nuts. When we correlated mother tree growth measurements with progeny isozyme allele frequencies by locus, none of the correlations were significant at the 5% level. However, we found a significant positive correlation at the 10% level between tree height and the frequency of a PGI2 allele. Using canonical correlation analysis, the results were more encouraging. The first canonical correlation was greater than 0.99 and the second one was 0.72. Diameter growth had a higher correlation with allele frequencies than height growth. Canonical correlation may be more practical than simple correlation analysis for studying relationships between isozyme allele frequencies and growth variables because of the polygenic inheritance pattern of growth traits.

Keywords: Juglans nigra L., electrophoresis, isozyme.

INTRODUCTION

Chemical composition of enzymes is determined by DNA strands in chromosomal genes; therefore, variation in chemical and/or physical enzyme composition reflects genetic variation. Electrophoresis can separate different proteins and enzymes on the basis of ionic charge, size, and shape. Separation occurs on gel media in response to variable enzyme migration rates at a given electric field. Interpretation of isozyme banding is based on the segregation patterns scored for enzyme polymorphism.

A preliminary study of isozyme variation in black walnut (*Juglans nigra* L.) was initiated in 1984 to determine which isozyme systems are best suited for discriminating among black walnut genotypes (Rink *et al.* 1989). We assayed 21 enzyme systems and found eight polymorphic enzymes which provided eleven loci with detectable isozyme variation: AAT1, ACO1, ACO2, PGM1, PGM2, PGM3, 6-PG2, ADH, FEST, ACP2, and PGI2. Another nine systems showed enzyme activity but lacked consistently reproducible results and were considered to be unsuitable. Those enzymes include DIA, GDH, BATA-GAL, GPT, G-2-D, EST, UGPP, SDH, and SKD. Four systems were apparently homozygotic (IDH G-6-P, ME, and MDH) and were also not useful.

---

[1] Professor, Dept. of Forestry, Southern Illinois University, Carbondale, IL. 62901, Principal Research Geneticist, USDA Forest Service, North Central Forest Experiment Station, Carbondale, IL. 62901, and Lecturer, Dept. of Forestry, Guangxi Forestry College, Nanning, Guangxi, People's Republic of China.

Knowing the relationship between isozyme variation and fitness traits (growth, survival and reproduction) variation may improve the efficacy and efficiency of selective breeding. Potentially superior trees may be identified by isozyme banding pattern. The allele arrangement of superior trees might indicate whether pure lines or hybrid lines in certain enzyme systems should be maintained. For example, Werner and Moxley (1991) found a relationship between malate dehydrogenase (MDH) isozyme genotype and plant vigor in peach [*Prunus persica* (L.) Batsch]. Homozygous Mdh1-2/Mdh1-2 individuals showed the greatest vigor, and were significantly different in vigor from Mdh1-1/Mdh1-1 homozygotes and from Mdh1-1/Mdh1-2 heterozygotes.

Instead of comparing the distribution of height growth among known fixed genotypes as proposed by Werner and Moxley (1991), we explored the correlation between height growth and isozyme frequency. Although correlation and causation are not synonymous, the use of correlation may serve us as a tool for expanded exploratory data analysis.

METHODS

Average height and diameter of black walnut trees in two progeny test plantations in southern Illinois were measured at age 19. Seed from fast and slow growing families were collected in 1989. Height, in decimeters, and diameter at breast height, in millimeters, are presented in Table 1. The average number of trees measured was 10, and the average number of nuts collected was 29 per family.

Table 1. Growth and isozyme allele frequency of 13 black walnut families. The top nine families were fast growing and the bottom four were slow growing.

FAM no.	HT dm.	DBH mm.	PGM21	PGM22	ACO11	ACO21	ACO22	6PG21	6PG22	FEST1	ACP21	PGI21
6300	94	128	0.50	0.50	0.50	0.75	0.25	0.75	0.25	1.00	1.00	1.00
6342	93	155	0.67	0.29	0.63	0.73	0.15	0.76	0.24	1.00	0.68	0.95
6344	84	100	0.81	0.17	0.68	0.61	0.39	0.64	0.25	0.94	0.58	0.98
6303	81	103	0.45	0.55	0.67	0.82	0.16	0.92	0.04	0.99	0.76	1.00
6325	76	119	0.75	0.25	0.45	0.82	0.15	0.93	0.07	0.98	1.00	0.96
6340	74	113	0.68	0.32	0.52	0.80	0.20	0.68	0.32	1.00	1.00	0.84
6325	72	98	0.57	0.43	0.80	0.53	0.47	0.90	0.10	0.69	0.69	0.80
6394	70	106	0.82	0.18	0.78	0.60	0.40	0.66	0.14	1.00	0.97	1.00
6340	65	118	0.70	0.30	0.53	0.69	0.18	0.65	0.12	0.95	0.92	1.00
6386	62	97	0.75	0.20	0.84	1.00	0.00	0.69	0.31	1.00	1.00	1.00
6382	61	92	0.58	0.40	0.53	0.53	0.45	0.59	0.41	0.99	0.58	0.95
6391	61	91	0.60	0.40	0.50	1.00	0.00	1.00	0.00	1.00	0.97	0.71
6386	52	75	0.40	0.60	0.60	1.00	0.00	0.40	0.60	1.00	0.50	0.60

Nuts were dehusked and stored at 2-5°C until needed, then cracked open and embryos removed. Embryos from each nut were individually macerated in a leaf extraction buffer described by Marty *et al.*(1984). Filter paper wicks (2 x 10

mm.) were soaked with the resulting liquid and then inserted in the starch gel. Electrophoretic methods used in this study are described by Marty *et al.* (1984).

Interpretation of isozyme banding was on the basis of segregation patterns. Embryo genotype data were analyzed using the Multilocus Estimation Program of Ritland and Jain (1981). Independent and variable allele frequencies in progeny are presented in Table 1.

STATISTICAL ANALYSIS

The CORR procedure (SAS 1982), the RSQUARE and the CANCORR procedures (SAS 1985) were used for data analysis. The CORR procedure computes Pearson product moment correlation between variables. The RSQUARE procedure finds subsets of independent variables that best predict a dependent variable by linear regression. We defined allele frequencies as independent and height or diameter as dependent variables. The CANCORR procedures analyzes the relationship between two sets of variables. Each set can contain several original variables. Our variable set named "growth" contained measurements on height and diameter, while the variable set "gene" contained allele frequencies.

Because of the low number (n=4) of slow growing families, we found that the relationship between growth and allele frequency was not significantly different from that of the fast growing families. Therefore, the two sets were pooled together as a single data base.

RESULTS AND DISCUSSION

Because the model used for the RSQUARE and the CANCORR procedures must be a full rank model, not all observed allele frequencies can be included. Therefore, we eliminated allele frequencies which were fixed. Fixation may be due to limited sample size or statistical dependency between alleles for a given locus. For example, we found the allele frequency ADH1 = 1.0 in each of the 13 families. In the previous study the allele frequency ADH1 =0.923 and ADH2 =0.077 among 948 embryos from 26 open-pollinated progenies (Rink *et al.* 1989). To avoid statistical dependency, we systematically excluded the last allele frequency in each locus. For example, PGM2 was interpreted to have three alleles: PGM21 PGM22 and PGM23, the PGM23 allele became fixed once the other two were included. Therefore, PGM21 and pgm22 were used but PGM23 was not studied.

None of the correlations between growth and allele frequency in Table 2 were significant at the 5% level. However, at the 10% level we found a positive correlation between the frequency of a PGI2 allele and growth. Taller and bigger mother trees tended to produce embryos with greater PGI2 allele frequencies.

Phosphoglucose isomerase (PGI) is known to be an enzyme which catalyzes the interconversions of glucose-6-phosphate and fructose-6-phosphate, both important substrates for respiration. However, the exact role alleles PGI1 and PGI2 have in this process or in the accumulation of biomass for height and diameter in black walnut trees is not known.

In most cases, the pattern of correlations of allele frequencies with height growth and with diameter growth were similar. We interpreted the algebraic sign

Table 2. Correlation analysis for growth and allele frequency.

isozyme locus	Between allele frequency and	
	Ht.	Dbh.
	Correlation Coefficients	
PGM21	0.1193	0.2763
PGM22	-0.1261	-0.2910
ACO11	-0.0661	-0.2066
ACO21	-0.3206	-0.2196
ACO22	0.2651	0.0451
6PG21	0.3384	0.2562
6PG22	-0.3077	-0.2851
FEST1	-0.0085	0.1350
ACP21	0.1113	0.3035
PGI21	0.5405	0.5325

Table 3. R-square of regression models for dependent variable HT and for dependent variable DBH

	R-square	Variables in HT Model
1	0.292	PGI21
2	0.364	6PG21 PGI21
3	0.416	6PG21 6PG22 PGI21
4	0.452	ACO21 6PG21 6PG22 PGI21
5	0.472	ACO21 ACO22 6PG21 6PG22 PGI21
6	0.482	ACO21 ACO22 6PG21 6PG22 FEST1 PGI21
7	0.497	PGM21 PGM22 ACO21 ACO22 6PG21 6PG22 PGI21
8	0.513	PGM21 PGM22 ACO21 ACO22 6PG21 6PG22 ACP21 PGI21
9	0.522	PGM21 PGM22 ACO11 ACO21 ACO22 6PG21 6PG22 ACP21 PGI21
10	0.538	PGM21 PGM22 ACO11 ACO21 ACO22 6PG21 6PG22 FEST1 ACP21 PGI21

	R-square	Variables in DBH Model
1	0.283	PGI21
2	0.429	ACO21 ACO22
3	0.572	ACO21 ACO22 ACP21
4	0.597	ACO21 ACO22 ACP21 PGI21
5	0.705	ACO21 ACO22 6PG21 6PG22 ACP21
6	0.742	ACO21 ACO22 6PG21 6PG22 ACP21 PGI21
7	0.765	ACO11 ACO21 ACO22 6PG21 6PG22 FEST1 ACP21
8	0.767	ACO11 ACO21 ACO22 6PG21 6PG22 FEST1 ACP21 PGI21
9	0.789	PGM21 PGM22 ACO11 ACO21 ACO22 6PG21 6PG22 FEST1 ACP21
10	0.805	PGM21 PGM22 ACO11 ACO21 ACO22 6PG21 6PG22 FEST1 ACP21 PGI21

of the correlation as an indicator of vigor. For example, in a two allele system, fast growing families displayed greater frequencies of PGI21, ACP21, and ACO12; and slow growing families had greater frequencies of PGI22, ACP22 and ACO11. Similarly, in a 3-allele system, fast growing was associated with more PGM21, ACO22 and 6PG21, and slower growth with more PGM22 ACO21 and 6PG22. However, there was some indication that increasing FEST1 frequency is associated with increased diameter but not height growth.

Because growth of forest trees is considered to be under the control of many additive genes, it is logical that variation in growth may potentially be modelled using a combination of several isozyme systems. The RSQUARE procedure calculates R^2 for all possible combinations of subset variables. However, from the given 10 loci we can construct 10 regression models using one locus, 45 models for 2 loci, 120 models for 3 loci. It is not necessary to report every possible model here, so only the top ones are presented in Table 3.

Using greater numbers of enzyme systems results in a better fit of the regression. Tree diameter can be predicted better than tree height from the allele frequencies in the multiple regression model. When all 10 frequencies were used, the R-square for the DBH model was 0.805, but for the HT model, it was only 0.538.

Because simple and multiple correlation are special cases of canonical correlation in which one or both sets contain a single variable, we wanted to explore the general relationship between growth and allele frequency in greater detail. Although the 10 allele frequencies may be statistically independent but biologically correlated variables as are tree heights and tree diameters, canonical analysis enabled us to extract canonical variates that are uncorrelated to each other and which can produce the highest correlation between growth and allele frequency. Since the number of canonical variables always equals to the smaller number of the original variables in the two sets, we had two canonical variables from each set. We named the canonical variables extracted from the 10 alele frequencies as GENE1 and GENE2, and those extracted from height and diameter as GROWTH1 and GROWTH2.

The first canonical correlation between GROWTH1 and GENE1 was 0.99, and the second canonical correlation between GROWTH2 and GENE2 was 0.72 (Table 4). The canonical variable GROWTH1 is associated mainly with diameter, and GROWTH2, with height. Therefore, the factor GROWTH1 may be associated with cambial growth and GROWTH2, with shoot growth. The canonical variable GENE1 is associated mainly with allele PGM21, PGM22, ACO11 and ACP21, while GENE2 is associated with ACO21, ACO22, 6PG21, 6PG22, and PGI21. Although the genetic factors GENE1 and GENE2 are likely responsible for biochemical functions common to isozyme systems in each group, exact interpretation of these genetic factors awaits the availability of a larger data base.

The relationship between simple correlation and canonical correlation can be explained as follows. According to path analysis (Li, 1975), correlation between two variables is the sum or product of all paths between them: paths arranged in series are multiplicative while paths in parallel are additive. For example, there are two routes from PGI21 to DBH: one goes through GENE1-GROWTH1, the other goes through GENE2-GROWTH2. Therefore, the correlation between PGI21

and DBH is

        0.2982*0.9942*0.7854+0.6765*0.7156*0.6190= 0.2328+0.2997=0.5325

which is in agreement with the simple correlation given in Table 2. Similarly,
the simple correlation between PGI21 and HT can be calculate from the canonical
structure diagram through the routes of GENE1-GROWTH1 and GENE2-GROWTH2:

        0.2982*0.9942*0.2367+0.6765*0.7156*0.9716=0.0702+0.4703=0.5405

Table 4. Canonical correlation analysis and canonical structure between
growth and allele frequency.

Canonical Correlation Analysis

Canonical Variables	Canonical Correlation	Approx. Standard Error
GROWTH1-GENE1	0.9942	0.0033
GROWTH2-GENE2	0.7156	0.1408

Canonical Structure

Set original variable	Correlation with their own canonical variables		Correlation with the opposite canonical variables	
	GROWTH1	GROWTH2	GENE1	GENE2
HT	0.2367	0.9716	0.2353	0.6953
DBH	0.7854	0.6190	0.7809	0.4430
	GENE1	GENE2	GROWTH1	GROWTH2
PGM21	0.3175	0.0641	0.3157	0.0459
PGM22	-0.3338	-0.0684	-0.3319	-0.0490
ACO11	-0.2608	-0.0069	-0.2593	-0.0050
ACO21	-0.0245	-0.4527	-0.0243	-0.3240
ACO22	-0.1961	0.4477	-0.1950	0.3204
6PG21	0.0643	0.4650	0.0640	0.3328
6PG22	-0.1411	-0.3949	-0.1403	-0.2826
FEST1	0.2227	-0.0877	0.2214	-0.0628
ACP21	0.3687	0.0353	0.3666	0.0253
PGI21	0.2982	0.6765	0.2965	0.4841

    Although the above two simple correlations are similar in size (0.5325 vs.
0.5405), and in paths (GENE1-GROWTH1 and GENE2-GROWTH2), their components are

263

quite different. The route from GPI21 toward DBH via GENE1-GROWTH1 contributed 0.2328/0.5325=44% to the simple correlation, but only 0.0702/0.5405=13% toward HT. Thus, the canonical correlations, GENE1-GROWTH1 and GENE2-GROWTH2, are the common links between any genetic variables on one side and any growth variables on the other side. They are the most important components and principal paths. However, the simple correlation is also determined by the strength between the set variables and their own canonical variable.
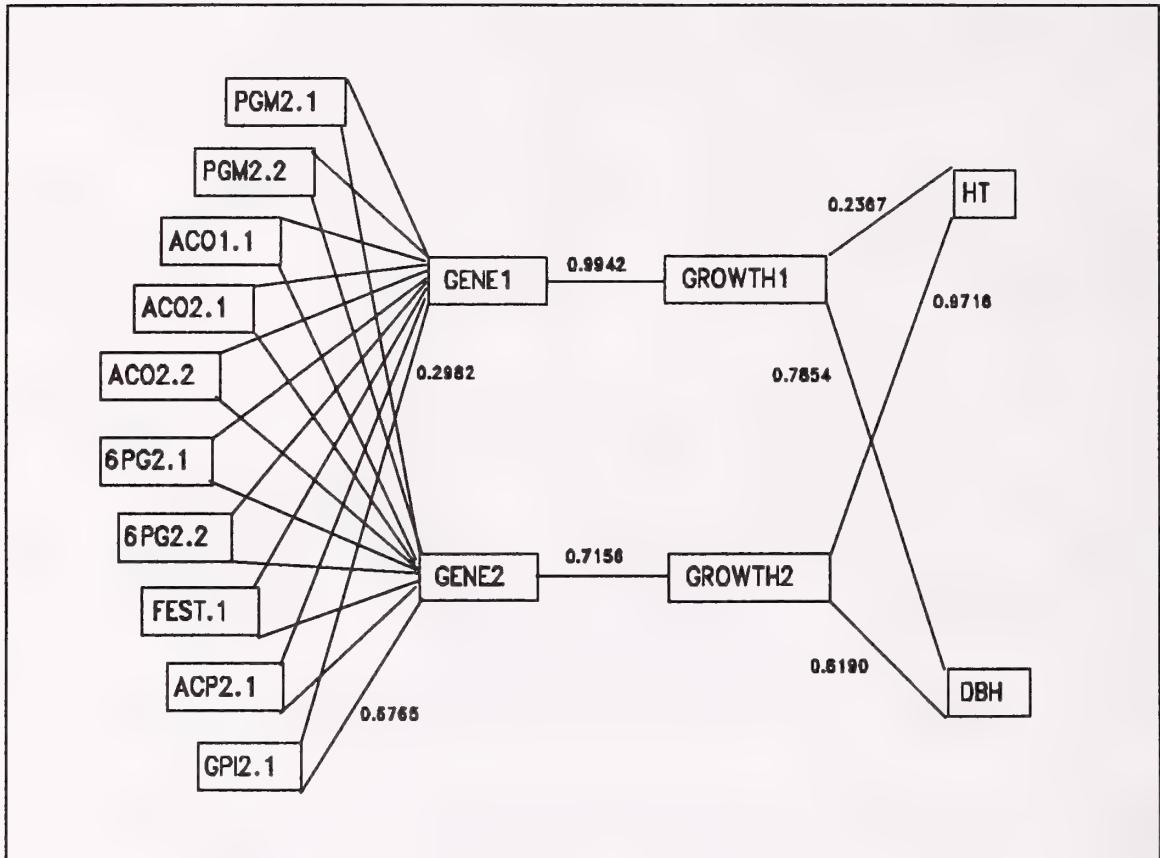
Figure 1. Path diagram and canonical structure for allele frequencies on the left and growth traits on the right.

CONCLUSIONS

Variation in height and diameter growth of black walnut trees seems to be related to phosphorylated enzyme frequencies. Diameter growth has a higher correlation with allele frequencies than height growth. Canonical correlation analysis is a better statistical tool to study the relationship between vigor and isozyme frequency than simple or multiple correlation.

LITERATURE CITED

Li,C.C. 1975. Path Analysis - a primer. The Boxwood Press, Pacific Grove. Calif. 346 pp.

Marty,T.L., D.M.O'Malley, and R.P.Guries. 1984. A manual for starch-gel electrophoresis: A new microwave edition. Univ. Wisc. Staff. Pap. Ser. No. 20. 23 pp.

Rink,G., E.R.Carroll, and F.H.Kung. 1989. Estimation of Juglans nigra L. mating system parameters. For. Sci. 35(2):623-627.

Ritland,K., and S.Jain. 1981. A model for the estimation of outcrossing rate and gene frequencies using N independent loci. Heredity. 47:35-52.

SAS. 1982. SAS User's Guide: Basics 1982 edition. SAS Inst. Inc. Cary. NC. 923 pp.

SAS. 1985. SAS User's Guide: Statistics. Version 5 ed. SAS Inst. Inc. Cary. NC. 957 pp.

Werner,D.J., and D.F.Moxley. 1991. Relationship between Malate Dehydrogenase isozyme genotype and plant vigor in peach. J. Amer. Soc. Hort. Sci. 116(2):327-329.

RELATING THE SEED COAT OF PINUS TO SPEED OF GERMINATION, GEOGRAPHIC VARIATION, AND SEEDLING DEVELOPMENT

James P. Barnett

Abstract.--Loblolly pine (Pinus taeda L.) evaluations indicate that speed of germination, which refects dormancy, is directly related to the ratio of the weight of the seed coat to total seed dry weight. Further evaluations with loblolly and ponderosa pine (P. ponderosa Dougl. ex Laws.) show significant correlations between the ratio of seed coat weight to total seed weight and ecotypic variation and seedling development. Seed dormancy was shown to vary by geographic location and to influence seedling development if stratification treatments are not optimized for conditions under which germination occurs. This finding may result in the maternal effects of the seed coat obscuring other genetically controlled growth processes early in seedling development. The effect of these early seed coat differences on seedling development can be minimized by extending the length of seed stratification.

Keywords: Pinus taeda, Pinus ponderosa, seed dormancy, seed weight, genetic variation.

INTRODUCTION

The influence of seed size and weight on early seedling growth of tree species has been known for over 50 years (Baldwin 1942, Champion 1928, Gast 1937). Righter (1945) found that in the genus Pinus, the positive correlation between seed weight and seedling height was temporary and disappeared after time in the field. A more recent study with loblolly pine (Pinus taeda L.) has shown a statistically significant positive correlation between seed weight and tree height after 15 years (Robinson and van Buijtenen 1979). Khalil (1981) reported that seed weight in white spruce (Picea glauca [Moech] Voss) was positively connected with annual growth of the terminal shoot at 2 to 4 years.

Several studies have evaluated the effect of size and other seed properties on germination and early seedling development. The evidence that seed size alone is a useful criterion to predict seedling performance continues to be conflicting (Belcher and Gresham 1974, Barnett and Dunlap 1982, Wrzesniewski 1982). Other seed parameters that may be closely related to size are probably more directly related to seed and seedling performance. Dunlap and Barnett (1983) found that larger loblolly pine seeds germinated more quickly and produced larger germinants than smaller ones after 28 days. Size differences resulted from differences in the rate

---

of germination unique to each seed size class. Seedling size and possibly uniformity of growth were considered a function of germination patterns that were strongly influenced by seed size and weight. Results from a number of studies have shown that germination rates (Barnett 1979, Dunlap and Barnett 1984, McLemore 1969) and subsequent seedling growth (Barnett and McLemore 1984, Boyer et al. 1985) can be manipulated in pines by means of seed stratification procedures. Seed stratification affects rates of germination of dormant seeds and, in turn, affects early seedling development.

Therefore, parameters that are determintal to or closely related to rates of germination may provide a better means of predicting early seedling performance than seed weight or size alone.

REVIEW OF SEED COAT-GERMINATION RELATIONSHIPS

The relationship of the ratio of seed coat weight to total dry seed weight was evaluated in a number of southern pine species with a wide range of dormancy (Barnett 1976). This work showed that as much as 69 percent of the variation in speed of germination in five southern pine species was related to seed coat weight as a proportion of total seed dry weight. Speed of germination was expressed as days to reach peak value--the mean daily germination of the most vigorous component of the seed lot (Czabator 1962). This relationship was supported by evidence that constraint by the seed coats and megagametophytes is directly related to dormancy. Measurements of water absorption indicated that seed coats restricted water uptake by limiting how much the megagametophyte and embryo could expand. Loblolly pine seeds, the most dormant of the tested seeds, attained only about 36 percent moisture content (dry weight basis) until the seed coats cracked and germination began. In contrast, longleaf pine (Pinus palustris Mill.) seeds, the least dormant of the tested seeds, never completely stopped imbibition and attained 55 percent moisture content before germination began. Changes in size of the megagametophyte, with and without seed coats, support the theory that seed coats restrict imbibition by preventing swelling and limiting water absorption in the more dormant seeds.

Respiration also followed the trends of moisture imbibition (Barnett 1976), and the patterns appeared to result from imbibition levels rather than impermeability to oxygen. Germinability of decoated seeds after different lengths of imbibition with seed coats intact and in atmospheres with various oxygen concentrations also supported the hypothesis that the seed coats slow germination by restricting megagametophyte and embryo expansion (Barnett 1972).

The total seed weight is determined by the seed coat, megagametophyte, and the embryo. As the weight of the seed coat increases, the proportional weights of the embryos of total weight decreases (table 1). For five southern pines--longleaf, Sonderegger (P. Xsondereggeri H. H. Chapm.), shortleaf (P. echinata Mill.), slash (P. elliottii Engelm.), and loblolly--the correlation coefficient was -0.930 (Barnett 1976). The same

relationship for five different ecotypes of ponderosa pine (P. ponderosa Dougl. ex Laws.) was computed from Anantachote's (1980) data to be -0.915. Because the two parameters (weights of seed coats and embryos) are closely related, seed coats were used in the present evaluations because they were easier to measure.

Table 1.--<u>Proportions of the seed parts to total dry weight and corresponding germination data for southern pine seeds (adapted from Barnett 1976)</u>

Species	Proportion of seed parts			Germination data		
	Seed coat	Gametophyte	Embryo	Total germination	Germination value	Peak day
	-------------percent-------------------					-no-
Longleaf	29.2	60.2	10.6	91	44.8	6.0
Sonderegger	35.1	55.5	9.4	97	43.4	7.4
Shortleaf	35.0	55.8	9.2	92	22.0	10.0
Slash	43.5	49.9	6.6	94	25.2	9.8
Loblolly	56.4	37.4	6.2	98	24.1	12.5

The close correlation between speed of germination and the ratio of the seed coat to total seed weight provides a means of rapidly estimating relative seed dormancy. The technique may more reliably estimate innate or true dormancy than seed germination tests, particularly in lots of stored seeds. Secondary dormancy, can be induced in pine seeds by unfavorable conditions during processing and storage (McLemore and Barnett 1966, 1968) and by adverse light and temperature regimes (McLemore and Hansbrough 1970, McLemore 1966), and secondary dormancy may mask the innate dormancy of seed.

RELATING SEED COATS TO ECOTYPIC VARIATION

Progeny tests with many coniferous species show that 60 to 90 percent of the variation in seedling size is closely related to maternal factors (Perry 1976). The seed characteristics of pines and other gymnosperms are largely derived from female tissue because only the embryo contains genes from both the pollen or male parents. Thus, it should be expected, that seed coat properties are related to seedling performance. The early expression of these maternal traits may affect the measurement of other genetic responses.

Loblolly pine seed lots from across the range of the species were evaluated to assess the variation in seed properties. Seed weight was unrelated to either latitude or longitude of the source (table 2). However, seed coat weight--expressed as ratio of seed coat weight to total seed weight--was positively correlated to latitude and negatively correlated to longitude. If seed coat thickness is directly related to dormancy or speed of germination, the degree of dormancy in loblolly seeds

268

should increase in the northern and eastern portion of the range and should decrease in the southern and western portion of the range. Thorbjornsen (1961) evaluated loblolly pine seed coat thickness and found thin seed coats in the western part of the range and thicker ones in the eastern part of the range.

Table 2.--Relation of geographic seed source of half-sib families of loblolly pine to seed weight and proportion of the seed coat to total dry weight

Location of seed source			Avg. seed weight[*]	Proportion of seedcoat to total seed weight[+]
County-State	Lat.	Long.		
			---mg---	-------percent--------
Cherokee, TX	31 21'	94 40'	32	54
Grant, AR	34 25'	92 20'	25	57
Lawrence, AL	34 30'	87 20'	36	60
Jackson, NC	35 15'	83 05'	30	62
Hertford, NC	36 25'	77 50'	26	63

[*]No statistically significant relationship was found between seed source and seed weight.

[+]Correlation coefficients between latitude and longitude and proportion of the coat to total seed weight were 0.94 and -0.96, respectively. Data are based on three replications of 50 seeds each.

Anantachote (1980) also evaluated ponderosa pine seedling development for a wide range of seed parameters and ecotypic selections; however, he did not attempt to relate the ratio of seed coat or embryo weight to total seed weight to geographic distribution or seedling development. A reevaluation of this ponderosa pine data shows a relationship very similar to that of loblolly pine. Percentages of the seed coat weight to total seed weight range from 39 to 53.2 and are negatively related to embryo weight (table 3). Correlations of seed coat weight as a proportion of total weight, with locations within each ecotype of ponderosa pine, provided some interesting relationships (table 4). The proportion of the seed coat was significantly related to longitude and elevation of the seed source (-0.96 and 0.89, respectively). No relationship was found with latitude of the source. However, when the product of latitude and elevation was evaluated, a positive correlation coefficient of 0.94 was obtained. Thus, seed dormancy was greater at the higher elevations in the interior portion of the range (Fig. 1). The coastal sources were less dormant.

RELATING SEED COATS TO SEEDLING DEVELOPMENT

Anantachote (1980) provides the best data relating the ratio of the seed coat to total seed weight to seedling development. He determined the growth of the primary root system of ponderosa pine seedlings grown in glass-sided boxes in a greenhouse environment. Root elongation was measured at 2 and 9 months (table 3). At 2 months, root length was negatively related to the ratio of the seed coat weight to total weight ($r = -0.957$) (table 4). However, at 9 months, no significant correlation was obtained. The same associations were determined with shoot length at 2 and 9 months. Correlation coefficients of -0.796 and -0.935 were found, relating shoot length at 2 and 9 months to ratio of the seed coat of total seed weight (table 4).

Table 3.--Relationship of geographic seed source of half-sib ponderosa pine families to seed characteristics and seedling development (developed from Anantachote 1981)

Ecotype[*]	Location of ecotypic source			Proportion of total wt.[+]		Seedling development			
	Lati-tude	Longi-tude	Eleva-tion	Seed coat	Embryo	Primary root length		Shoot length	
						2 mos.	9 mos.	2 mos.	9 mos.
			--m--	---percent---		------------------cm--------------			
A-California	35 5'	120 2'	1,524	41.5	6.5	69.8	86.0	7.8	15.5
B-No. plateau	44 8'	118 5'	1,348	39.0	7.5	70.0	81.8	6.6	14.2
C-So. interior	36 0'	113 0'	2,134	47.0	6.0	65.5	85.9	4.5	11.5
D-Cent. interior	37 2'	105 7'	2,165	53.2	4.0	64.7	84.0	4.8	9.0
E-No. interior	44 5'	105 5'	1,913	51.0	5.4	65.5	74.7	4.6	8.3

[*] The five ecotypes of ponderosa pine (Wells 1963) and the location of the sample stands: A-California, B-Idaho, and Oregon, C-Arizona, D-Colorado and New Mexico, and E-South Dakota and Wyoming.

[+] Seed characteristics were determined by measuring five randomly selected seeds from each of 16 half-sib families. The number of family selections in each ecotype were: A-2, B-2, C-3, D-4, and E-5.

Seedling characteristics were determined by measuring two plants from each family in each of three groups of boxes grown under greenhouse conditions.

These data may indicate that seeds that are less dormant and germinate faster also begin root and shoot development sooner. However, the data are not sufficiently well documented to determine if speed of germination was definitely related to seedling growth.

Table 4.--<u>Correlation coefficients relating proportion of ponderosa pine seed coats of total seed weight, geographic location, and seedling development (from Anantachote 1981)</u>

Variables correlated with proportion of seedcoat of total seed weight	Correlation coefficients[*]
Proportion of embryo of total weight	-0.915
Latitude of ecotypic sources	-0.287
Longitude of ecotypic sources	-0.959
Elevation of ecotypic sources	0.892
Latitude times elevation	0.935
Primary root length (2 months)	-0.957
Primary root length (9 months)	-0.314
Shoot length (2 months)	-0.796
Shoot length (9 months)	-0.935

[*]A value of $\pm$ 0.878 is necessary for statistical significance at the 0.05 level.

DISCUSSION

Although significant correlations do not necessarily reflect causal relationships, when evaluated with other biologically sound data, they are important indicators of biological responses. Earlier research has established that dormancy or speed of germination in southern pines is related to embryo constraint by the seed coat and megagametophyte (Barnett 1972, 1976; Carpita et al. 1983). This relationship probably holds for other <u>Pinus</u> species. Recent research has also shown that larger loblolly pine seeds produce larger seedlings primarily because they germinate more promptly (Dunlap and Barnett 1983).

Stratification of seeds usually results in faster germination, which is why stratified seeds usually produce larger plants than unstratified seeds. When stratified and unstratified seeds germinate on the same date, stratification has no affect on development (Barnett and McLemore 1984). A few days difference in time of germination may significantly affect seedling development (Boyer et al. 1985). Therefore, it is easy to understand how differences in seed dormancy may affect seedling

development. Short periods of stratification may seem to eliminate these differences in rate of germination when evaluations are made under standard laboratory conditions. However, when germination occurs in the field or on nursery beds where conditions are less than optimum, the rate of germination is markedly reduced, and seedlings from late germinating seeds tend to produce inferior quality plants because of competition from previously established seedlings (McLemore 1969, Dunlap and Barnett 1984).

Seed dormancy in loblolly and ponderosa pine varies ecotypically with northern and eastern sources, and higher elevations have greater dormancy. This variation may also occur with other pine species. Particularly with ponderosa pine, a species that has a wide range of geographic diversity (Wright 1976), this variation in dormancy probably reflects the differences in precipitation, temperature, and day-length at the seed source. These trends probably reflect natural selection; i.e., if seeds germinate too early, they may be killed by frost and, if too late, by competition for light and moisture from earlier seedlings (Campbell and Ritland 1982). The response of seeds to environmental cues during dormancy should tend to maximize fitness by optimizing the timing of germination (Levins 1969).

Maternal factors such as seed coat properties that influence the speed of germination can obscure the nature of genetic control of subsequent growth processes (Perry 1976). Less than 15 percent of the weight of a conifer seed is in the embryo, which is the only portion with a genetic component from the male parent. In nature, stratification is usually optimized as a result of natural conditions, but in nursery production, the genetic component from the male parent may be obscured when researchers do not optimize the stratification needs of the seed lot. Seed dormancy varies by geographic location or ecotype, and stratification procedures should be designed to meet the needs of each ecotype. These stratification needs should be determined under the stress conditions that relate to nursery bed conditions where seeds are to be sown. However, the stratification period can be extended to minimize the effect of the seedcoat on initial seedling development.

LITERATURE CITED

Anantachote, Anan. 1980. Geographic variation of root development of ponderosa pine (Pinus ponderosa Laws.) seedlings as related to shoot growth and seed characteristics. Doctoral dissertation, University of Idaho, Moscow, Idaho. 44 p.

Baldwin, H.I. 1942. Forest tree seed of the north temperate regions with special reference to North America. Chronica Botanica Co., Waltham, Massachusetts 240 p.

Barnett, James P. 1972. Seed coat influences dormancy of loblolly pine seeds. Canadian Journal of Forest Research 2:7-10.

Barnett, James P. 1976. Delayed germination of southern pine seed related to seed coat constraint. Canadian Journal of Forest Research 6:504-510.

Barnett, James P. 1979. Germination temperatures for container culture of southern pine. Southern Journal of Applied Forestry 3:13-14.

Barnett, James P., and James R. Dunlap. 1982. Sorting loblolly pine orchard seeds by size for containerized seedling production. Southern Journal of Applied Forestry 6:112-115.

Barnett, James P., and B.F. McLemore. 1984. Germination speed as a predictor of nursery seedling performance. Southern Journal of Applied Forestry 8:157-162.

Belcher, F.W., Jr., and H.H. Gresham. 1974. Seed sizing: benefit or detriment. Pages 117-121 in Proceedings of the Southeastern Nurserymen's Conference, 1974 August 6-8; Gainesville, FL. Atlanta, GA: USDA Forest Service, State and Private Forestry.

Boyer, James N., David B. South, Carl Muller, Harry Vanderbeer, Walker Chapman, and William Rayfield. 1985. Speed of germination affects diameter at lifting time of nursery-grown loblolly pine seedlings. Southern Journal of Applied Forestry 9:126-135.

Campbell, Robert K., and Stanley M. Ritland. 1982. Regulation of seed-germination timing by moist chilling in western hemlock. New Phytologist 92:173-182.

Carpita, N.C., A. Skaria, J.P. Barnett, and J.R. Dunlap. 1983. Cold stratification and growth of radicles of loblolly pine (Pinus taeda) embryos. Physiologia Plantarum 59:601-606.

Champion, H.G. 1928. The effect of size of germination and development of seed of sal (Shorea robusta). Indian Forestry 54:93-96.

Czabator, F.J. 1962. Germination value: an index combining speed and completeness of pine seed germination. Forest Science 8:386-396.

Dunlap, J.R. and J.P. Barnett. 1983. Influence of seed size on germination and early development of loblolly pine (Pinus taeda L.) germinants. Canadian Journal of Forest Research 13:40-44.

Dunlap, J.R., and J.P. Barnett. 1984. Manipulating loblolly pine (Pinus taeda L.) seed germination with simulated moisture and temperature stress. Pages 61-74 in M.L. Duryea and G.N. Brown, eds. Seedling physiology and reforestation success. Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, Netherlands.

Gast, P.R. 1937. Studies on the development of conifers in raw humus. Statens Skogsfjorsokanst. (Sweden). Meddel 29:589-632.

Khalil, M.A. 1981. Correlation of juvenile height growth with cone morphology and seed weight in white spruce. Silvae Genetica 30:179-181.

Levins, R. 1969. Dormancy as an adaptive strategy. Symposia of the Society for Experimental Biology 23:1-10.

McLemore, B.F. 1966. Temperature effects on dormancy and germination of loblolly pine seed. Forest Science 12:284-289.

McLemore, B.F. 1969. Long stratification hastens germination of loblolly pine seed at low temperatures. Journal of Forestry 67:419-420.

McLemore, B.F., and J.P. Barnett. 1966. Loblolly seed dormancy influenced by cone and seed handling procedures and parent tree. Res. Note SO-41. New Orleans LA: U.S. Department of Agriculture, Forest Service, Southern Forest Experiment Station, 4 p.

McLemore, B.F., and J.P. Barnett. 1968. Moisture content influences dormancy of stored loblolly pine seed. Forest Science 14:219-221.

McLemore, B.F., and T. Hansbrough. 1970. Influence of light on germination of Pinus palustris seed. Physiologia Plantarum 23:1-10.

Perry, Thomas O. 1976. Maternal effects on the early performance of tree progenies. Pages 473-481 in M.G.R. Carrnell and F.T. Last, eds. Tree physiology and yield improvement. Academic Press, New York.

Righter, F.I. 1945. Pinus: The relationship of seed size and seedling size to inherent vigor. Journal of Forestry 43:131-137.

Robinson, John F., and J.P. van Buijtenen. 1979. Correlation of seed bed traits with 5, 10, and 15-year volumes in a loblolly pine progeny test. Forest Science 35:591-596.

Thorbjornsen, Eyvind. 1961. Variation patterns in natural stands of loblolly pine. Pages 25-44 in Proceedings of the Sixth Southern Forest Tree Improvement Conference; 1961 June 7-8; Gainesville, FL. U.S. Department of Agriculture , Forest Service, Southern Forest Experiment Station, New Orleans, LA. Gainesville, FL: Southern Forest Tree Improvement Committee.

Wells, O.O. 1963. Geographic variation in ponderosa pine. I. The ecotypes and their distribution. Silvae Genetica 13:89-103.

Wright, Jonathan W. 1976. Introduction to forest genetics. Academic Press, New York. 463 p.

Wrzesniewski, W. 1982. Physiology of Scots pine seedlings grown from
    seeds of different weight. III. Differentiation of seedlings grown
    during the first growing season. Acta Physiologie Plantarum
    4:139-151.

FIGURE 1.-The five ecotypes of ponderosa pine (Wells 1963) and the
locations of the sample stands. A--California (sample stand 1), B--north
plateau (sample stands 2 and 3), C--southern interior (sample stands 4 and
5), D--central interior (sample stands 6, 7, and 8), E--northern interior
(sample stands 9 and 10) (adapted from Anantachote 1981)

CHLOROPLAST DNA VARIATION IN SHORTLEAF, SLASH, LONGLEAF, AND LOBLOLLY PINES

W. L. Nance[1], C. D. Nelson[2], D. B. Wagner[3], T. Li[4], R. N. Patel[5], and D. R. Govindaraju[6]

Abstract.--We have identified a chloroplast DNA polymorphism that separates shortleaf, slash, longleaf, and loblolly pines into three groups. Although little intraspecific variation was evident, the marker was polymorphic in slash pine. This marker may be useful for monitoring interspecific hybridization and introgression in the southern pines.

---

[1] Project Leader, Genetics and Pathology of Southern Pine Forest Ecosystems, Southern Forest Experiment Station, Gulfport, Mississippi.

[2] Research Scientist, Genetics and Pathology of Southern Pine Forest Ecosystems, Southern Forest Experiment Station, Gulfport, Mississippi.

[3] Assistant Professor, Department of Forestry, University of Kentucky, Lexington, Kentucky.

[4] Graduate Research Assistant, Department of Forestry, University of Kentucky, Lexington, Kentucky.

[5] Principle Laboratory Technician, Department of Forestry, University of Kentucky, Lexington, Kentucky.

[6] Research Associate, Department of Forestry, University of Kentucky, Lexington, Kentucky. Present address: Department of Biology, Case Western Reserve University, Cleveland, Ohio.

INTRODUCTION

Recently, chloroplast DNA (cpDNA) has been added to the list of markers that are useful in the investigation of interspecific hybridization in conifer populations, because cpDNA markers often distinguish congeneric species (Wagner et al. 1987, Szmidt et al. 1988, Govindaraju et al. 1989, Wang and Szmidt 1990). The predominantly paternal mode of inheritance of coniferous chloroplast genomes (Ohba et al. 1971, Neale et al. 1986, Szmidt et al. 1987, Wagner et al. 1987, Chesnoy 1987, Neale and Sederoff 1988, Szmidt et al. 1988, Neale and Sederoff 1989, Neale et al. 1989, Stine et al. 1989, Wagner et al. 1989, Stine and Keathley 1990, Neale et al. 1991) may permit novel insights into the mechanisms and consequences of natural hydridization in conifers. Unfortunately however, the potential applications of cpDNA markers have not been fully explored in the economically important pines of the southeastern USA (but see Neale and Sederoff 1989, Ali et al. 1991).

We are interested in using cpDNA as a marker to study hydridization and possible introgression in the southern pines (for example, in longleaf x loblolly pine) and have recently identified a cpDNA polymorphism in shortleaf, slash, longleaf, and loblolly pines. Here we present survey data for this polymorphism.

MATERIAL AND METHODS

We surveyed 195 individuals from a research plantation of the Southwide Southern Pine Seed Source Study (Wakeley 1953) in Pearl River County, Mississippi. This included 45 individuals from 7 populations of shortleaf pine, 32 individuals from 6 populations of slash pine, 40 individuals from 6 populations of longleaf pine, and 78 individuals from 14 populations of loblolly pine.

The methods for total cellular DNA purification, restriction endonuclease digestion, gel electrophoretic fractionation of restriction fragments, and transfer of restriction fragments to Biotrans membranes (blots) have been described previously (Southern 1975, Murray and Thompson 1980, Wagner et al. 1987, Sambrook et al. 1989). Chloroplast DNA BamHI fragments on blots were hybridized with a ^{32}P-labeled restriction fragment cloned from the chloroplast genome of lodgepole pine (Pinus contorta Dougl.), and visualized by autoradiography (Southern 1975, Feinberg and Vogelstein 1983, Lidholm et al. 1988).

RESULTS AND DISCUSSION

The cpDNA polymorphism was nearly monomorphic within shortleaf, longleaf, and loblolly pines (Table 1), and the four unusual individuals in these three species may have resulted from interspecific hybridization. In contrast, four genotypes were detected in slash pine, none of which occurred in any sample from the other three species. Four of the six slash pine populations were polymorphic, with three of these populations containing at least three genotypes.

The polymorphism provided a species-specific marker for slash pine in our samples, while the cpDNA distinctions among shortleaf, longleaf, and loblolly pines were less distinct (Table 1). Nonetheless, since cpDNA variant frequencies appear to

distinguish longleaf from both loblolly and slash pines, it is potentially valuable for use in screening non-grass stage phenotypes in longleaf pine nursery beds. However, the actual utility of cpDNA markers in screening material for longleaf pine breeding programs can be determined only by further testing.

Table 1. Chloroplast DNA variant numbers in four pine species.

Chloroplast DNA variant[a]	Numbers of each variant			
	Shortleaf	Slash	Longleaf	Loblolly
2.8/10.5	42	0	40	1
2.5/4.0	3	0	0	77
2.5/7.9	0	7	0	0
2.5/10.5	0	22	0	0
2.5/4.0/10.5	0	2	0	0
2.5/4.0/7.9	0	1	0	0
Total	45	32	40	78

[a]Variants are denoted by the sizes (in kilobase pairs, kbp) of variable restriction fragments. The probe was a 6.4 kbp BamHI fragment, extending in the lodgepole pine chloroplast genome from 16S to psbC/psbD (J. Lidholm, pers. comm; Strauss et al. 1988).

ACKNOWLEDGEMENTS

LITERATURE CITED

Ali,I.F., D.B.Neale, and K.A.Marshall. 1991. Chloroplast DNA restriction fragment length polymorphism in Sequoia sempervirens D. Don Endl., Pseudotsuga menziesii (Mirb.) Franco, Calocedrus decurrens [Torr.] Florin, and Pinus taeda L. Theor. Appl. Genet. 81:83-89.

Chesnoy,L. 1987. L'origine des organites du cytoplasme embryonnaire chez les gymnospermes. Bull. Soc. Bot. Fr., Actual Bot. 134:51-56.

Feinberg,A.P., and B.Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. 132:6-13.

Govindaraju,D.R., B.P.Dancik, and D.B.Wagner. 1989. Novel chloroplast DNA polymorphism in a sympatric region of two pines. J. Evol. Biol. 2:49-59.

Lidholm,J., A.Szmidt, and P.Gustafsson. 1988. Lack of one rDNA repeat and duplication of psbA in the chloroplast genome of lodgepole pine (Pinus contorta Dougl.). P.67-74 in Molecular genetics of forest trees, W.M.Cheliak and A.C.Yapa (eds.). Can. For. Serv. Inf. Rep. PI-X-80, Chalk River, Ontario.

Murray,M.G., and W.F.Thompson. 1980. Rapid isolation of high molecular weight plant DNA. Nucl. Acids Res. 8:4321-4325.

Neale,D.B., and R.R.Sederoff. 1988. Inheritance and evolution of conifer organelle genomes. P.251-264 in Genetic manipulation of woody plants, J.W.Hanover and D.E.Keathley (eds.). Plenum Press, New York, New York.

Neale,D.B., and R.R.Sederoff. 1989. Paternal inheritance of chloroplast DNA and maternal inheritance of mitochondrial DNA in loblolly pine. Theor. Appl. Genet. 77:212-216.

Neale,D.B., K.A.Marshall, and D.E.Harry. 1991. Inheritance of chloroplast and mitochondrial DNA in incense-cedar (Calocedrus decurrens). Can. J. For. Res. 21:717-720.

Neale,D.B., K.A.Marshall, and R.R.Sederoff. 1989. Chloroplast and mitochondrial DNA are paternally inherited in Sequoia sempervirens D. Don Endl. Proc. Natl. Acad. Sci. USA 86:9347-9349.

Neale,D.B., N.C.Wheeler, and R.W.Allard. 1986. Paternal inheritance of chloroplast DNA in Douglas-fir. Can. J. For. Res. 16:1152-1154.

Ohba,K., M.Iwakawa, Y.Okada, and M.Murai. 1971. Paternal transmission of a plastid anomaly in some reciprocal crosses of Sugi, Cryptomeria japonica D. Don. Silvae Genet. 20:101-107.

Sambrook,J., E.F.Fritsch, and T.Maniatis. 1989. Molecular cloning: a laboratory manual, second edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Southern,E.M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Molec. Biol. 98:503-517.

Stine,M., and D.E.Keathley. 1990. Paternal inheritance of plastids in Engelmann spruce X blue spruce hybrids. J. Hered. 81:443-446.

Stine,M., B.B.Sears, and D.E.Keathley. 1989. Inheritance of plastids in interspecific hybrids of blue spruce and white spruce. Theor. Appl. Genet. 78:768-774.

Strauss,S.H., J.D.Palmer, G.Howe, and A.H.Doerksen. 1988. Chloroplast genomes of two conifers lack an inverted repeat and are extensively rearranged. Proc. Natl. Acad. Sci. USA 85:3898-3902.

Szmidt,A.E., T.Aldén, and J.-E.Hällgren. 1987. Paternal inheritance of chloroplast DNA in Larix. Plant Molec. Biol. 9:59-64.

Szmidt,A.E., Y.A.El-Kassaby, A.Sigurgeirsson, T.Aldén, D.Lindgren, and J.-E.Hällgren. 1988. Classifying seedlots of Picea sitchensis and P. glauca in zones of introgression using restriction analysis of chloroplast DNA. Theor. Appl. Genet. 76:841-845.

Wagner,D.B., G.R.Furnier, M.A.Saghai-Maroof, S.M.Williams, B.P.Dancik, and R.W.Allard. 1987. Chloroplast DNA polymorphisms in lodgepole and jack pines and their hybrids. Proc. Natl. Acad. Sci. USA 84:2097-2100.

Wagner,D.B., D.R.Govindaraju, C.W.Yeatman, and J.A.Pitel. 1989. Paternal chloroplast DNA inheritance in a diallel cross of jack pine (Pinus banksiana Lamb.). J. Hered. 80:483-485.

Wakeley,P.C. 1953. Progress in the study of pine races. South. Lumberman 187:137-140.

Wang,X.-R., and A.E.Szmidt. 1990. Evolutionary analysis of Pinus densata (Masters), a putative Tertiary hybrid. 2. A study using species-specific chloroplast DNA markers. Theor. Appl. Genet. 80:641-647.

SEASONAL VARIATION IN METABOLIC RATE AND ITS CORRELATION TO HALF-SIB FAMILY PERFORMANCE IN LOBLOLLY PINE

S. R. Wann[1], J. E. Yakupkovic[1], W. R. Goldner[2] and G. A. Lowerts[3]

Abstract.--Measurement of metabolic rate by heat conductance calorimetry (HCC) has been previously shown to predict long-term growth performance in larch and coastal redwood. In order to demonstrate the validity of this technique as a selection tool in loblolly pine, the metabolic rate of six seed orchard clones of known height performance level (PL) was measured throughout the growing season using a differential scanning calorimeter operating in the isothermal mode. Metabolic rates varied during the 1990 growing season, with rates highest just prior to the spring growth flush and lowest during high summer. Metabolic rates began to increase in mid-August and rose to levels comparable to those measured in early spring. The late season increase in metabolic rate was coincident with (1) the initiation and development of female strobili primordia and (2) and additional cycle of shoot growth with the relief from the droughty conditions that persisted through most of the spring and summer of 1990. When metabolic rates were measured right before the first flush of growth in the spring, the rate was found to correctly rank four of five clones sampled and only the order of the two lowest height PL clones was switched. Particularly noteworthy in this ranking was that the height PL data was for the progeny of the seed orchard clones while the metabolic rate measurements were made on the parents. Therefore it appears that metabolic rate measurements can be used to identify good seed orchard clones, as well as outstanding individuals for vegetative propagation. The dependence of metabolic rate on the environment, the developmental status of vegetative shoots and the differences in clone phenology with respect to shoot growth cycles suggests that genetic comparisons should only be made when trees are in the same physiological condition.

Keywords: Pinus taeda L., heat conductance calorimetry, metabolic rate, genetic selection, height performance level.

INTRODUCTION

Inasmuch as the rotation age of loblolly pine (Pinus taeda L.) can be several decades long, advanced generation selection has been focused on evaluating juvenile performance characteristics

---

[1] Senior Research Scientists, Union Camp Corporation, Research and Development Division, Princeton, NJ 08543.

[2] Research Scientist, Union Camp Corporation, Research and Development Division, Princeton, NJ 08543.

[3] Research Geneticist, Union Camp Corporation, Forest Resources Division, Bellville, GA 30414.

in progeny tests. Selection typically occurs 4-8 years after progeny test establishment. Several early selection experiments have attempted to reduce the selection age further with varying results. Some of the more promising experiments in early selection have examined iterative growth processes such as shoot growth cycles (Bridgwater, 1990), and nutritional considerations (Li et al, 1989).

The contributions of nutritional and physiological parameters to growth suggest that techniques for early selection may be developed that are independent of growth measurements. Such "laboratory" techniques would concentrate on processes intrinsic to the cells or tissues in a tree and would not require measurements of field performance. It has been forwarded (Hansen et al., 1989) that the obvious candidates for laboratory tests of intrinsic growth processes (photosynthetic rates and carbon dioxide uptake rates) have not been successful predictors of future performance because these processes are seldom rate limiting to growth. On the other hand, if the rate limiting step to growth is the incorporation of fixed carbon dioxide into biomass, then a measure of the metabolic rate should be predictive of the growth potential.

Measurement of metabolic rates (by HCC) in larch (Hansen et al., 1989) and coastal redwood (Hart Scientific Co., 1989) have been previously correlated to long-term growth potential in these species. In order to investigate the application of this technique to loblolly pine, metabolic rates were measured throughout the season on seed orchard clones of known height PLs.

MATERIALS AND METHODS

Plant Material

Study trees were selected from a second-generation loblolly pine seed orchard established ca. 1976 near Savannah, GA. Clones studied represented selections of known height PL. Height PL was determined from open-pollinated progeny tests using the methods of Hatcher et al (1981). For metabolic rate measurements recorded throughout the season, three clones were chosen as "poor" performers (height PL = 25 ± 6) and three as "good" performers (height PL = 79 ± 2). Shoot tips were collected from the same ramet in each clone, and all ramets were located in the same block of the orchard. Shoot tips were collected from the upper 25% of the crown. Care was taken to avoid shoots that were damaged by insects or disease. Shoot tips were wrapped in moist paper towels and shipped on ice overnight for HCC. Shoot tips could be stored at $4°C$ for up to 12 days prior to metabolic rate measurements.

In an experiment aimed at investigating the effect of shoot development status on metabolic rate, fourteen seed orchard clones encompassing the entire range of performance level were analyzed in mid-May, 1990. At the time of sampling, shoot tips were placed into four categories with respect to the degree of shoot elongation. The four categories were: (1) a dormant, or "winter" type bud, (2) a winter type bud that appeared to be on the verge of flushing, (3) shoot tips in which the needle fascicles had elongated less than 15 mm ("pinfeather" stage) and (4) shoot tips in which needle fascicles had elongated more than 15 mm.

Heat Conductance Calorimetry

HCC was conducted using a Du Pont model 910 Differential Scanning Calorimeter operating in the isothermal ($27.0°C$) mode. In order to accommodate a loblolly pine shoot tip, custom stainless steel sample holders were constructed by Chatam Precision (Union, NJ). The cylindrical sample holders had a threaded lid that was fitted with a neoprene rubber gasket. Using this sample holder,

shoot tips approximately 7 mm in length, with a fresh weight on the order of 40-70 mg, were analyzed.

All metabolic rate determinations were performed at least in triplicate, and each determination required about 20 min to reach equilibrium. Since it often took several days day to perform HCC analysis for all the samples from a particular collection date, analysis of the first clones sampled was repeated to confirm that no sample degradation was occurring over the period of analysis. All samples were run double-blind; neither the HCC operator or the person preparing the sample knew the identity of the samples under analysis.

RESULTS AND DISCUSSION

Figure 1 shows a typical time course for metabolic rate determinations. The direction of approach to equilibrium (the curved portion of Figure 1) is from negative heat fluxes reflecting the fact that since the metabolic rate was measured at temperatures greater than ambient, heat had to first flow into the sample to return to the measurement temperature. The absolute difference in the two flat portions of the Figure 1 (the baseline is on the left), expressed in microwatts (μW), is the heat produced by the sample and is taken as the metabolic rate.
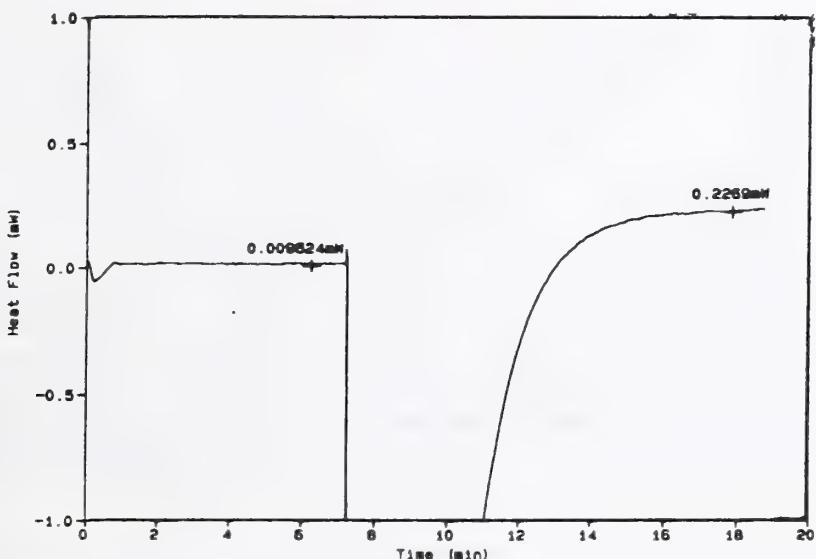
Figure 1. Sample data plot for calorimetric measurement of metabolic rate of loblolly pine shoot tips (see text for explanation).

Metabolic rates reported here for loblolly pine averaged about 5-10 μW/mg throughout the growing season are in agreement with values reported for other conifers. For example, metabolic rates of balsam fir and larch recorded in the summer are both reported to be about 15 μW/mg (Hansen et al., 1989), and those for coastal redwood about 1.0 μW/mg (Hart Scientific Co., 1989).

Figure 2 shows the variation in metabolic rate for the high and low height PL clones for a 194 day period beginning March 6, 1990. Note that at no time throughout this period was the metabolic rate of the high height PL clones significantly ($p = 0.05$) greater than the low height PL clones. However, statistically significant differences were observed in the metabolic rates for different sampling dates. Metabolic rate started out highest just prior to the spring growth flush, but diminished after that until it reached a minimum in mid-July. Metabolic rate began to increase

in August and reached values comparable to those observed in the spring. Rates then remained high throughout the fall, but dropped dramatically in late-October to levels below 1.0 µW/mg (data not shown). The increase in metabolic rate in August was coincident with two observations: (1) relief from the droughty conditions that had persisted since March, and (2) the differentiation of female cone primordia that were observed in shoot tip sections by September (Greenwood, 1980).
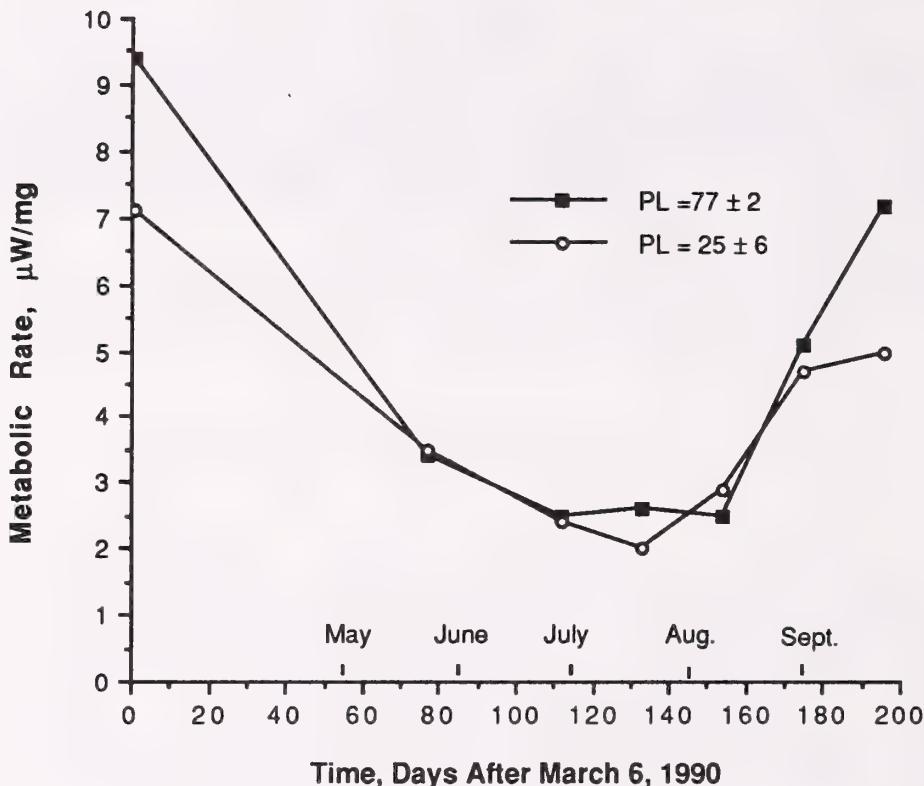
Figure 2. Seasonal variation in metabolic rate of loblolly pine for high and low height PL seed orchard clones. (For a given date, differences between high and low height PL clones were not significant as determined by independent t-test. Before May and after September, means were significantly different by paired t-test.)

Indictment of the hot, dry conditions that persisted near Savannah during the 1990 growing season as the reason for the decline in metabolic rate is evident in Table 1. In 1990, during the three month period (March-June) rainfall totalled only 3.77" and the metabolic rate dropped significantly for both good and poor performing genotypes. In 1991, abundant precipitation was recorded at the seed orchard and no significant reduction in metabolic rate has been observed from March to June for both good and poor performing clones. From what is known of meristematic activity in pine shoot apices, it would be expected that metabolic rates would rise through spring and peak in summer as long as conditions were favorable for growth (Cannell et al., 1976). These

observations suggest that prior to correlating metabolic rates to growth potential, consideration should be given to environmental factors that can influence metabolic rate.

Table 1. Comparison of metabolic rates of loblolly pine seed orchard clones in two consecutive years of high and low rainfall (means followed by common letters are not significantly different as determined by Tukey's HSD).

Year	Rainfall, in (Mar.-June)	Mean Height PL	Metabolic Rate, µW/mg	
			Early Mar.	Late May
1990	3.77	77	9.4 a	3.4 b
		25	7.1 a	3.5 b
1991	20.49	77	9.1 a	8.4 a
		25	8.0 a	9.1 a

In addition to possible environmental and reproductive ontological influences, differences in the extent of shoot development in each clone was demonstrated to have an effect on metabolic rate. Although all the clones examined were chosen from the same geographic region, and steps were taken to minimize environmental variation among the ramets sampled, there was considerable variation in the rate of shoot elongation between clones. Therefore, at any particular sampling time (except during dormancy), shoots could be categorized into the four previously described groups, depending on the extent of shoot elongation. Among fourteen clones sampled during mid-May as the elongation of "summer" shoots (typically the second growth flush of the season) was beginning, significant differences in metabolic rates were observed between the various shoot types shown in Table 2. Shoots in the "pinfeather" stage had the highest metabolic rates, but it is of interest to note that these clones also had the lowest height PLs. In contrast, three of the four clones that were the farthest along in the elongation of the the first summer shoot were among the best clones in the orchard, but had the lowest metabolic rate at the time of sampling. Apparently, shoot tip metabolism during shoot and fascicle elongation may not be as intense as during the time of bud break.

Table 2. The influence of the extent of shoot elongation (collected 5/14/90) on the metabolic rates of fourteen loblolly pine seed orchard clones (within a column, means followed by common letters are not significantly different as determined by Tukey's HSD).

Shoot Elongation Class	Clone, No.	Mean Height PL	Mean Metabolic Rate, µW/mg
Dormant; between flushes	2	57 a	3.0 a
Swollen; about to flush	3	57 a	3.9 a
"Pinfeather stage"	5	32 b	6.4 b
Needle fascicles > 15 mm	4	65 a	1.7 a

The cosegregation of height PL and metabolic rate when shoots were classified according to their state of development may provide insight into the dynamics of shoot growth cycles and supplement early selection approaches based on shoot elongation patterns (Bridgwater, 1990). The observation that metabolic rate is a function of shoot development means that genetic comparisons should take into account variations in the extent of shoot elongation. Therefore, a single, common, sampling date for all clones in a study may not be possible.

Accurate identification of good and poor performing families is more important than the overall correlation coefficient for early selection (Carter et al., 1990). Bearing in mind that genetic comparisons should be restricted to trees that are in the same developmental point in a shoot cycle, Table 3 ranks five clones that were sampled just prior to the spring growth flush in descending order of metabolic rate. At the time of sampling in early March, these five clones still had an encased bud that had not yet begun to swell. In Table 3 it can be seen that ranking with respect to metabolic rate correctly identified the top three clones and only switched the order of the two clones with the lowest height PL. Particularly noteworthy in all these attempts to correlate performance with metabolic rate, was that while height PL is based on analysis of the progeny of seed orchard clones, metabolic rate determinations were made on the parents. Therefore it appears that metabolic rate determinations may identify good seed orchard clones, as well as outstanding individuals for vegetative propagation.

Table 3. Metabolic rate ranking of five loblolly pine seed orchard clones (sample date = 3/6/90).

Clone Height PL	Metabolic Rate, μW/mg
81	10.6
79	10.3
77	8.8
15	7.2
30	7.1

CONCLUSIONS

The results presented here are the first reported measurements of metabolic rates of loblolly pine. The value of metabolic rate as a selection tool holds considerable promise, especially due to the apparent relationship of metabolic rate to shoot growth cycles. In loblolly pine, shoot cycles, environmental stresses, and the differentiation of reproductive structures must be taken into consideration before genetic comparisons are made. Metabolic rate measurements may lead to a better understanding of the dynamics of shoot formation itself, perhaps by providing information on the intensity and persistence of metabolic activity during stem unit initiation. In addition, the sensitivity of metabolic rate to environmental stress that was strongly implicated here, and has been demonstrated elsewhere (Criddle et al., 1988), may prove useful in identifying trees that can maintain high metabolic rate under adverse conditions.

LITERATURE CITED

Bridgwater, F. E. 1990. Shoot elongation patterns of loblolly pine families selected for contrasted growth potential. Forest Sci. 36: 641-656.

Cannell, M. G. R., S. Thompson and R. Lines. 1976. An analysis of inherent differences in shoot growth within some northern temperate conifers. In: Tree Physiology and Yield Improvement. (eds.) M. G. R. Cannell and F. T. Last. pp 173-205.

Carter, K. K., G. W. Adams, M. S. Greenwood and P. Nitschke. 1990. Early family selection in jack pine. Can. J. For. Res. 20: 285-291.

Criddle, R. S., R. W. Breindenbach, E. A. Lewis, D. J. Eatough and L. D. Hansen. 1988. Effects of temperature and oxygen depletion on metabolic rates of tomato and carrot cell cultures and cuttings measured by calorimetry. Plant, Cell and Environment 11: 695-701.

Greenwood, M. S. 1980. Reproductive development in loblolly pine: I. The early development of male and female strobili in relation to the long shoot behavior. Amer. J. Bot. 67: 1414-1422.

Hansen, L. D., E. A. Lewis, D. J. Eatough, D. P. Fowler and R. S. Criddle. 1989. Prediction of long-term growth rates of larch clones by calorimetric measurement of metabolic heat rates. Can. J. For. Res. 19: 606-611.

Hart Scientific Company. 1989. Determining tree growth from calorimetric measurement of metabolic heat. Application Note. 4 p.

Hatcher, A. V., F. E. Bridgwater and R. J. Weir. 1981. Performance level - standardized score for progeny test performance. Silvae Genet. 30: 184-187.

Li, Bailian, S. E. McKeand and H. L. Allen. 1989. Early selection of loblolly pine families based on seedling shoot elongation characteristics. In Proc. 20th Southern. For. Tree Imp. Conf. Charleston, SC. pp. 228-235.

FIVE-YEAR EVALUATION OF LOBLOLLY PROGENY TESTS ESTABLISHED WITH BOTH BARE-ROOT AND CONTAINERIZED SEEDLINGS[1]

C. R. McKinley [1]

Abstract.--Two loblolly pine (Pinus taeda L.) progeny tests, planted with both containerized and bare-root seedlings, were evaluated after five years in the field. Containerized seedlings were consistently smaller. Survival differences by seedling type were not apparent in either test. Family rankings across the two seedling types were generally more closely correlated for height and diameter than for volume. In one test family rankings were highly correlated for the two seedling types. In the other test, family rankings were more consistent in drought-hardy sources than in sources selected for growth rate and form. The lack of significant family rank correlations for the two seedling types in the second test raises questions regarding family selection from containerized tests. One family, in particular, showed dramatic rank changes between bare-root and containerized material.

Keywords: Pinus taeda L., containerized seedlings, progeny testing, family selection.

INTRODUCTION

A considerable number of genetic tests have been and are continuing to be established with greenhouse-grown containerized seedlings. The advantages of using these seedlings for genetic tests are well presented by van Buijtenen and Lowe (1981). Tests established with containerized seedlings are generally assumed to provide source or family information consistent with that obtained from tests established from bare-root seedlings. However, little information is available to verify or disprove this assumption. Studies directed at performance of containerized vs bare-root seedlings offer little guidance as conflicting results has often been reported. For example, South and Barnett (1986) showed containerized loblolly pine (Pinus taeda L.) seedlings were taller than bare-root seedlings after three growing seasons on a relatively dry site. However, the bare-root seedlings were taller on a moist site. Goodwin (1976) reported containerized loblolly seedlings were taller than bare-root seedlings after three years in North Carolina. Conversely, Barnett (1981) showed greater height for bare-root seedlings after three years in Louisiana when March and April plantings were evaluated. Studies involving shortleaf pine (Pinus echinata Mill.) have also given different results depending upon site conditions (Ruehle et al., 1981), although Brissette and Barnett (1989) recommended using containerized material for shortleaf. Containerized longleaf (Pinus palustris Mill.) was reported to outperform bare-root material on a dry site in North Carolina (Goodwin, 1976).

The Texas Forest Service (TFS) has been utilizing container-grown greenhouse seedlings to establish genetic field tests for a number of years. In order to further examine the question of how containerized seedlings perform relative to bare-root seedlings, two loblolly pine tests were established at separate locations using both types of material. Results after five years of field growth ares reported in this paper. The objectives of this analysis are

---

1/ Associate Geneticist, Texas Forest Service, Texas A&M University, College Station, Texas

to 1) determine if significant growth differences exist between the two seedling types and 2) determine if family by seedling type interaction is of large enough magnitude to merit concern in selecting families from containerized-grown tests.

METHODS

Seedling Production

Seedlings for the two field tests were grown at different times using different material. Field test #221 was sown in the greenhouse in spring 1982, with bare-root seedlings grown during the 1982 season at the TFS Indian Mound Nursery. Nine control-pollinated families from both superior and drought hardy material were evaluated. Four of the nine crosses were reciprocals. Field test #238 was sown in the greenhouse in fall, 1984, with bare-root seedlings grown during the 1984 season at the Indian Mound Nursery. A total of twelve open pollinated sources were utilized with six characterized as drought hardy (based on previous TFS information) and six selected for growth rate and form (obtained from Louisiana, east of the Mississippi River). Containerized seedlings were grown in 163 cu.cm. commercially-available containers with a 1:1 mixture of peat and vermiculite. Supplemental heating and lighting were provided the fall sown seedlings. Bare-root seedlings were grown according to standard TFS nursery practices. Table 1 indicates the appropriate growing regime for each of the seedling types.

Table 1. General production schedule for bare-root (nursery) and containerized (greenhouse) seedlings.

Activity	Nursery	---------Greenhouse--------- Spring Sown	Fall Sown
Seed Stratification	February	March	September
Sowing	April	April	October
Fertilization	May-August	May-September	November-March
Outplanting	December-April	November	April

Field Plantings

Planting #221 was established in fall, 1982, while planting #238 was established in spring, 1985. Greenhouse schedules resulted in each type of seedling being planted at different times at each site. Both tests utilized a split-plot design (Snedecor and Cochran, 1967) with seedling type (containerized or bare-root) included in the main plots and families as sub-plots. Planting #221 is located near Alto, (Cherokee County) and consists of the nine sources with three replications and six tree row plots. Planting #238 is located near San Augustine (San Augustine County) and consists of the twelve sources with 12 replications and six tree row plots. Both sites are located in East Texas with the largest nearby town being Lufkin. The Alto site is approximately 83 km. west of San Augustine. The Alto site is located on a sandy loam soil, while the San Augustine site tends more toward a clay loam.

Plantings were measured for height and diameter after five years in the field. Volume per tree was calculated using total height and diameter. Average family volume also included dead or missing trees. Number of trees living at age

five provided survival information.

Tables 2 and 3 indicate the characteristics of the two plantings after five years. Survival was similar for the two seedling types in both plantings. However, in Planting #238, survival for drought-hardy sources was somewhat higher than for the growth rate and form (superior) sources (90 percent vs 82 percent). This difference dictated that the two groups be separated for further analysis of that planting, as growth traits (most notably, volume) are directly affected by number of living stems.

Table 2. Characteristics of plantation #221 for both bare-root and containerized seedlings after five years.

Trait	Bare-root	Containerized
Survival (%)	94.5	92.6
Height (m.)	5.26	4.32
Diameter (cm.)	7.81	6.05
Volume (cu. dm.)	8.55	4.35

Table 3. Characteristics of plantation #238 for both bare-root and containerized seedlings after five years.

Trait	----Superior Sources----		-Drought-Hardy Sources--	
	Bare-root	Containerized	Bare-root	Containerized
Survival (%)	81.3	82.8	90.5	89.0
Height (m.)	4.12	3.77	4.01	3.64
Diameter (cm.)	5.47	4.62	5.74	4.82
Volume (cu. dm.)	3.04	2.08	3.62	2.31

Statistical analysis (Tables 4, 5 and 6) show significant differences (prob.=.10 or less) between the two seedling types with only one exception (volume in Planting #221), with bare-root seedlings out-performing containerized trees. This result implies that growth characteristics can be expected to vary depending on whether seedlings are grown as containerized or bare-root. Family differences were also significant within each planting for the traits analyzed.

In evaluating the results of this analysis, the most critical question relates to the degree which family rankings are similar across the two seedling types. Selection of specific families in containerized tests should result in the same (or nearly so) families as those selected from bare-root tests of the same material. Since most artificial regeneration programs utilize nursery-grown seedlings, the 'correct' family ranking must be assumed to be that obtained from the bare-root tests. Containerized tests are, thus, actually utilized to predict the performance of those families if they had been grown as nursery seedlings.

Table 4. Results of split-plot analysis for five-year height, diameter and volume for plantation #221.

Source	d.f.	Height Mean Squares	F	Diameter Mean Squares	F	Volume Mean Squares	F
Main Plots							
Replication (R)	2	1.26	.25	5.84	.29	93.75	.56
Seedling Type (T)	1	66.58	13.53*	237.73	11.99*	1414.56	8.49
Error (RxT)	2	4.92		19.82		166.57	
Sub-Plots							
Cross (C)	8	3.54	5.21***	9.76	3.81***	49.94	2.84**
C x T	8	.21	.30	1.04	.41	11.27	.60
Error ((RxC)+(RxTxC))	32	.68		2.56		17.60	

* Indicates significance at .10 level of probability
** Indicates significance at .05 level of probability
*** Indicates significance at .01 level of probability

Table 5. Results of split-plot analysis for five-year height, diameter and volume of drought-hardy material in plantation #238.

Source	d.f.	Height Mean Squares	F	Diameter Mean Squares	F	Volume Mean Squares	F
Main Plots							
Replication (R)	11	5.3	4.22**	32.3	5.86**	72.1	3.76**
Seedling Type (T)	1	28.3	23.17***	163.0	29.75***	365.2	19.02***
Error (RxT)	11	1.2		5.5		19.2	
Sub-Plots							
Female (F)	5	1.6	3.10**	5.0	1.93*	12.4	2.09*
F x T	5	.1	.18	.62	.24	6.0	1.01
Error ((RxC)+(RxTxC))	110	.5		2.56		5.1	

* Indicates significance at .10 level of probability
** Indicates significance at .05 level of probability
*** Indicates significance at .01 level of probability

Table 6. Results of split-plot analysis for five-year height, diameter and volume of superior material in plantation #238.

Source	d.f.	Height Mean Squares	F	Diameter Mean Squares	F	Volume Mean Squares	F
Main Plots							
Replication (R)	11	2.0	.65	13.8	1.23	22.8	1.07
Seedling Type (T)	1	27.0	8.79**	142.6	12.63***	198.7	9.33**
Error (RxT)	11	3.1		11.3		21.3	
Sub-Plots							
Female (F)	5	3.2	4.50***	4.6	1.91*	14.8	2.54**
F x T	5	1.12	1.60	5.7	2.39**	10.7	1.84
Error ((RxC)+(RxTxC))	110	.7		2.4		5.8	

* Indicates significance at .10 level of probability
** Indicates significance at .05 level of probability
*** Indicates significance at .01 level of probability

Table 7 shows family rank correlations (Snedecor and Cochran, 1967) for the two plantations evaluated. Plantation #221 showed a high correlation between the two seedling types for all the three traits. Plantation #238 demonstrated significantly less family rank correlation than Planting #221 for both the superior and drought-hardy material. In both plantings, family rankings were more highly correlated for height and diameter than for volume. In Planting #238, ranking between the two seedling types tended to be more closely correlated for the drought-hardy sources than for the superior sources. Height ranking had a higher correlation than either diameter or volume, but in this test, only non-significant correlations were observed, and that higher correlation may not prove useful.

Table 7. Family rank correlation between bare-root and containerized seedlings after five years.

| Trait | Plantation #221 | ------------Plantation #238---------- | |
		Drought-Hardy Sources	Superior Sources
Height	.90**	.49	.43
Diameter	.90**	.60	.26
Volume	.77*	.43	.14

* Indicates significance at .05 level of probability
** Indicates significance at .01 level of probability

In most programs, families are selected on the basis of total volume. Thus, family ranking for volume for the two seedling types is the variable of interest. Figure 1 shows volume production for Planting #221. In this test, the better families generally performed well regardless of seedling type (as demonstrated by the family rank correlations). Separate statistical analysis indicated that significant differences occurred for volume among greenhouse-grown families (prob. = .009), but differences did not occur among nursery-grown families (prob.= .39). Therefore, family selection in this test, if based on seedlings grown in containers, would not adversely affect the amount of genetic gain which could be expected.
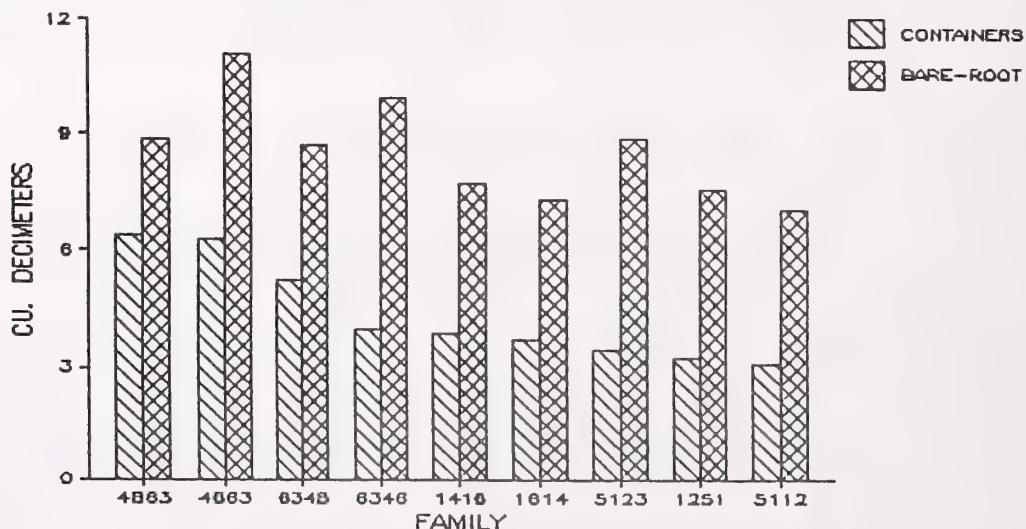

Figure 1. Five-year family volumes for containerized and bare-root seedlings for planting #221.

Planting #238 presents contrasting results relative to family selection from containerized tests, depending upon which group of families are considered (Figure 2). For the drought-hardy material, separate analysis indicated that significant volume differences occurred among families in the containerized-grown material (prob.= .005), but not among families in the nursery-grown material (prob.= .82). In the superior material, significant differences occurred among nursery grown-material (prob.= .011) but not in the containerized-grown families (prob.= .50). Selection in the drought-hardy sources would, thus, result in essentially the same scenario as presented above for Planting #221. From Figure 2, it can be observed that family 2015 (drought-hardy) ranked considerably different for the two seedling types, thus explaining a large part of the low family rank correlation for this material. Figure 2 also demonstrates why family rank correlation was low in the superior families. Source 3117 performed quite well in the bare-root part of the test but very poorly in the containerized plots. Additionally, this family was the only source shown to be significantly different from all other nursery-grown families (prob.=.05). Examination of data showed that survival for this family was the same for both greenhouse and nursery seedlings, and volume differences are consistent with both height and diameter differences.
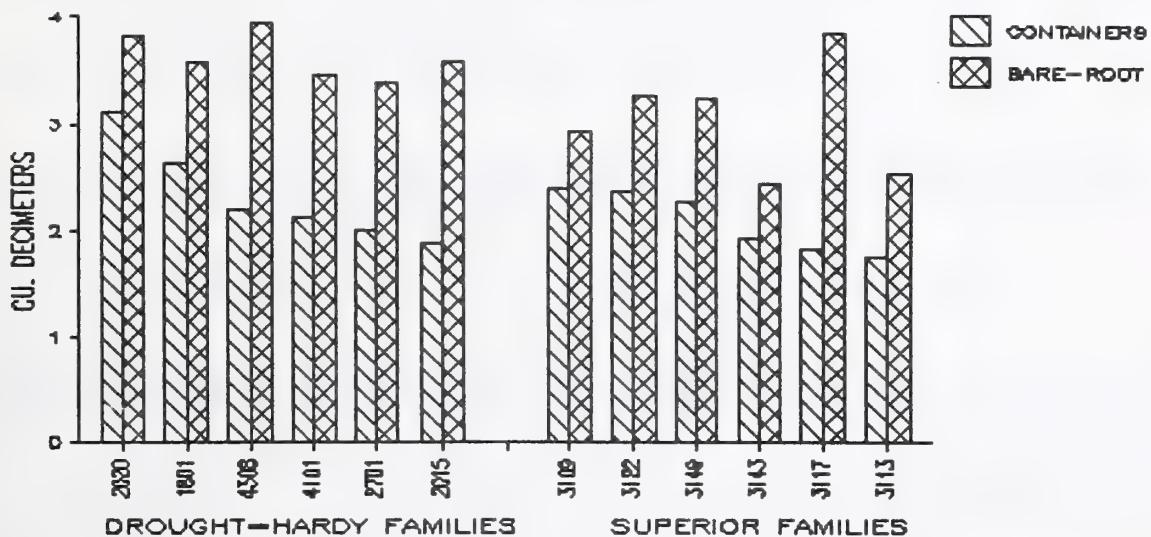
Figure 2. Five-year family volumes for containerized and bare-root seedlings for planting #238.

An argument can be made that non-significant family differences in the containerized material would preclude selection from within the superior sources, and thus differences in nursery-grown material would not result loss of genetic gain through family selection (as nursery-grown material is usually not established for progeny tests). However, the fact that one family in six performed so differently between the two seedling types should be of concern.

The data from this test is evidence that containerized-grown tests should be not be considered as totally representative of family performance regardless of seedling type. Several genetic testing procedures currently in practice, such as multiple tests in multiple locations, etc., provide some insurance against inaccurate selection. It is quite possible that additional measures (e.g. establishing tests using both seedling types) may be required to assure that family selection from genetic tests is most efficient.

CONCLUSIONS

Evaluation of five-year data of two loblolly progeny tests showed greater growth for seedlings established from bare-root material than from containerized

material. Whether differences will continue to exist is not known, but based on these results, it would seem advisable to modify growth and yield information to account for differences in seedling type.

Family differences within seedling types varied from test to test and from drought-hardy to superior sources. Family rank correlations ranged from relatively high in the Alto test to quite low for superior sources in the San Augustine test. Upon examination, one superior family in the San Augustine test was observed to make significant rank changes, thus having a major effect on the overall correlations.

Results presented here do not imply that greenhouse-grown progeny tests should not be used for selection purposes. Rather, they suggest that the possibility exists for some loss of genetic gain if family performance is affected by seedling type, and for at least one family, that effect was demonstrated.

LITERATURE CITED

Barnett, J.P. 1981. Selecting containers for southern pine seedling production. In Proc. Southern Containerized Forest Tree Seedling conference. Savannah, GA. P. 15-24.

Brissette, J.C. and J.P. Barnett. 1989. Comparing first-year growth of bare-root and container plantings of shortleaf half-sib families. In Proc. Southern Forest Tree Improvement Conf. Charleston, SC. P. 354-361.

Goodwin, O.C. 1976. Summer-planted loblolly and longleaf pine tubelings outgrow 1-0 nursery seedlings in North Carolina. Journal of Forestry. 74:515-516.

Ruehle, J.L., D.H. Marx, J.P. Barnett, and W.H. Pawk. 1981. Survival and growth of container-grown and bare-root shortleaf pine seedlings with Pisolithus and Telephora ectomycorrhizae. Southern Jour. of Applied Forestry. 5:20-24.

Snedecor, G.W. and W.G. Cochran. 1967. Statistical Methods. Sixth Ed. The Iowa State Univ. Press. Ames, Iowa. 593 p.

South, D.B. and J.P. Barnett. 1986. Herbicides and planting date affect early performance of container-grown and bare-root loblolly pine seedlings in Alabama. New Forests. 1:17-27.

van Buijtenen, J.P., and W.J. Lowe. 1981. Use of containerized seedlings for progeny testing. In Proc. Southern Containerized Forest Tree Seedling conference. Savannah, GA. P. 145-148.

IDEAL FIBERS FOR PULP AND PAPER PRODUCTS

R.L. Ellis[1] and A.W. Rudie[2]

Abstract.--The various paper and paperboard products are made for different purposes and therefore have different product specifications and standards. Printing applications require smooth, low porosity paper with sufficient strength to carry the mineral fillers and coatings needed to obtain opacity and gloss. Paperboard products need stiffness and compressive strength to perform well under stacking loads. As the paper requirements change, so do the preferred characteristics of the pulp fibers and the ability of the papermaker to adjust for unfavorable fiber form. Pulping processes also differ in ability to handle diverse tree species. In particular, the mechanical pulping processes are highly species dependent, favoring low-density softwoods with fine fibers and thin cell walls.

Performance requirements for typical paperboard and coated paper products are reviewed and the softwood fiber characteristics that maximize performance are identified. In addition, the influence of fiber morphology on the production and performance of mechanical pulps is considered.

Keywords: Fiber morphology, mechanical pulp, coated paper, paperboard, linerboard, spruce (*Picea*), western hemlock (*Tsuga heterophylla*), Douglas-fir (*Pseudotsuga menziesii*), southern pine (*Pinus*).

INTRODUCTION

Of the many parameters useful as a measure of product performance, board bending stiffness is probably of greatest interest to the construction industry, and paper breaking length or tensile strength is of most interest to the papermaker. In Figure 1, average bending modulus for boards cut from various softwoods (*Wood Handbook*, 1974) is graphed against breaking length (MacLeod, 1980) for kraft pulps derived from the same species. Species that routinely give fibers capable of forming strong papers generally have poor stiffness as solid lumber products. Of the four softwoods selected, the spruces, often considered ideal papermaking fibers, have the weakest bending modulus as solid lumber products.
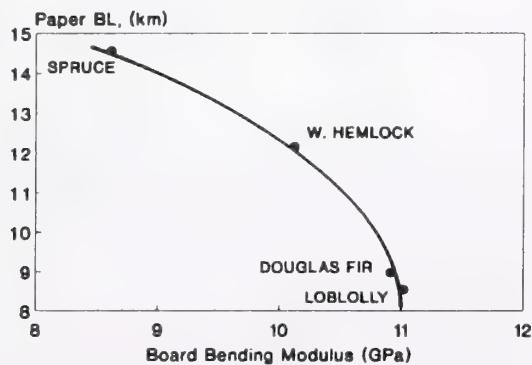

Figure 1. Paper breaking length graphed against board bending modulus for typical pulpwoods.

[1] Professor of Engineering, The Institute of Paper Science and Technology, Atlanta, GA.
[2] Assoc. Professor of Chemistry, The Institute of Paper Science and Technology, Atlanta, GA.

In an effort to highlight the needs and desires of the paper industry for wood fiber form, this paper will review the product requirements of various paper and paperboard grades and attempt to identify the key fiber characteristics that contribute to the manufacture of a superior product. To simplify the problem, the hardwood pulp contribution to paper and paperboard performance will not be considered in this paper, and the discussion will be limited to softwoods. The performance of the following representatives of four genera: spruce (white, black and Norway), western hemlock, Douglas-fir, and southern pine (loblolly and shortleaf) will be evaluated in various paper products. Table 1 summarizes average wood density and typical fiber characteristics for representatives of these four softwood genera.

Table 1. Fiber characteristics of common U.S. pulpwoods (Isenberg, 1980; Horn, 1972; Koch, 1972).

Species	Specific Gravity g/cc	Fiber Length mm	Fiber Diameter μm	Fiber Wall μm	Latewood Content %
Loblolly Pine	0.47	3.5-4.5	35-45	4-11	20-45
Douglas-fir	0.43	3.5-4.5	35-45	3-8	25
W. Hemlock	0.38	2.5-4.2	30-40	2-5	10-30
W. Spruce	0.37	2.5-4.2	25-35	2-3	3

MECHANICAL PULPING

In mechanical pulping, fiber characteristics are a dominant variable and exercise considerable control over the paper quality. Typical quality data for mechanical pulps from the four genera are presented in Table 2. The white wood and thin cell walls of spruce give the strongest and brightest mechanical pulp of the four, making it the preferred genus for high-yield pulping. Douglas-fir, giving low strength and low brightness, is rarely used in mechanical pulping.

Table 2. Typical pulp properties and energy consumptions of different species compared with spruce groundwood (Kurdin, 1980; Hatton and Cook, 1990).

| | Genus | | | |
	Spruce	W. Hemlock	Loblolly	Douglas-fir
Energy kWh/BDT	1900	1960	2500	2750
Freeness ml	120	80	100	100
Breaking Length km	4.8	3.7	3.3	3.4
Tear Index mNm2/g	9.7	8.3	7.3	6.3
Brightness	59	56	58	53

Traditionally, wood density has been the parameter most associated with differences in high-yield pulp quality between species. However, wood density does not control mechanical pulp quality but rather, some aspect of fiber structure that correlates well with wood density.

The Shallhorn, Karnis equation for the tensile strength of a bond limited paper domain such as newsprint, is given below (Shallhorn and Karnis, 1979).

$$T = \frac{N\pi r \, \Gamma l}{2}$$

where N is the number of fibers in the break, r is the fiber wall radius, Γ is the bond strength per unit fiber surface, l is the average fiber length.

All paper grades are made to a basis weight specification. Adjusting for basis weight by dividing by g/m^2 gives tensile index (or breaking length) and introduces the term N/g. Whereas basis weight is dictated by paper grade, N/g is controlled by fiber morphology. Average fiber weight can be calculated from fiber volume and density [$\pi r^2 - \pi(r-w)^2]l\rho$ where w is average fiber wall thickness and ρ is cell wall density.

Fiber length (l) cancels, cell wall density (Besley, 1969; Smith, 1965; Wangaard, 1969) (ρ) and bond strength per unit fiber surface area (Γ) are relatively constant for a given mechanical pulping process and within the softwoods of interest. The term m^2 introduced with basis weight is constant within a paper grade or a standardized test procedure. This leaves the term πr, which is 1/2 average fiber circumference, and $\pi r^2 - \pi(r-w)^2$, which is average fiber wall cross-sectional area (CSA).

$$TI = k \, \frac{\pi r}{CSA}$$

Using data from various literature sources, this ratio is graphed against TMP breaking length in Figure 2. The straight line obtained indicates that the ratio is a key factor in strength development of mechanical pulps. This result still needs to be evaluated using a coherent set of data.
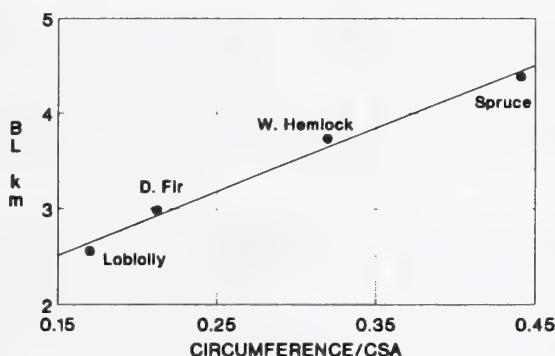
Figure 2. TMP breaking length at 2000 kWh/BDT specific energy graphed against the ratio of average fiber circumference divided by average fiber wall cross-sectional area.

COATED PRINTING PAPERS

The key performance requirements of coated printing papers are high smoothness, low porosity and high paper surface strength.

Smoothness and Porosity

To obtain high gloss and even print density on the coated paper, the final surface must be very smooth (Bristow and Ekman, 1981). The clay coating layer on a sheet of paper is on the order of 5μm thick (Kartovaara, 1989), comparable to the double-wall thickness of the average spruce fiber and half the double-wall thickness of a loblolly pine fiber. Although the coating process fills in the surface roughness of the sheet with the slurry coating clay, shrinkage on drying reproduces the original surface topography in reduced scale. Additional smoothness is gained by calendering the coated paper. The calendering process improves smoothness and unprinted sheet gloss but can also create other problems. Calendering can cause ink to absorb at different rates (Kartovaara, 1989) and can reduce sheet strength and opacity.

To obtain both high smoothness and low porosity in the base paper, papermakers prefer fibers with thin cell walls that collapse on drying to form ribbon-like fibers that conform to the surface of the other fibers in the sheet. This increases paper density, decreases porosity and assures that the maximum surface defect is on the order of 1 double-wall thickness, about 5 μm for spruce. Fibers with thick cell walls resist collapse. A cylindrical fiber is unable to conform to the other fibers in the paper, opening up the paper structure and increasing porosity. If a southern pine latewood fiber on the paper surface fails to collapse on pressing and drying, it can protrude above the average surface of the paper by one whole fiber diameter, 25 to 50 μm (Koch, 1972) and 5 to 10 times the average coating thickness.

Surface Strength

The heatset web offset printing process is a torture test for coated papers. Starting with the low porosity base paper, the coating reduces the porosity even further. In the offset printing process, water is used to protect the non-image part of the printing blanket so the paper picks up moisture in the press. After printing, the paper is dried rapidly in an oven to set the inks. The water in the paper turns to steam, which is restricted from expanding and escaping by the low porosity of the paper and the coating. The result is an internal force working to blow the sheet apart.

In lightweight coated papers containing mechanical pulps, the steam contributes to fiber rise, reforming the lumens in previously collapsed fibers. In lightweight coated papers with 100% chemical pulp in the base paper, the weak link is generally the interface between the paper and the coating that leads to coating blisters, much like paint blisters. Improved surface strength reduces the severity of both fiber rise and heatset printing blisters (Perry, 1972).

Good paper surface strength is favored by a high bonded surface between fibers. Bonded surface can be increased by mechanically tearing fibrils from the surface of the fibers. Fibers with a large surface area to mass also help. In studies of fibers readily pulled from the surface of papers containing mechanical pulp, heavy walled latewood fibers invariably dominate (Mohlin, 1989). As with smoothness and porosity, species with thin fiber walls, such as the spruces and the true firs, are preferred.

Manufacturers of lightweight coated printing papers prefer the lower density softwoods, primarily the spruces and true firs. Over 70% of the coated papers manufactured in the United States are produced in the northeast and north-central states where white and black spruce and balsam fir are available (Table 3).

Table 3. Pulp and paper production by region (Statistics, 1990).

| | % of U. S. PRODUCTION | | |
	South	N.E./N.C.	West
Total Paper	53%	31%	14%
Newsprint	58	11	31
Coated	22	71	4
Uncoated Free	40	45	12
Bleached Board	89	0	11
Kraft Board	82	0	17
Market Pulp	67	16	16

BLEACHED PAPERBOARD

Bleached paperboard is used in folded cartons and liquid packaging applications. Paperboard used in consumer packaging is a store display item and, for these applications, the board is coated to improve the printing characteristics. The requirements for printed folding carton applications are similar to those for lightweight coated papers, but the heavier basis weights and thicker clay coatings provide added flexibility for handling difficult fibers.

Stiffness

The other major requirement of packaging board is stiffness (Grangård, 1970). High board stiffness improves stacking strength and product protection. For liquid packaging applications it reduces carton bulge (Bridger and Munday, 1969) and makes the carton easier to hold.

Bending stiffness in a solid bleached paperboard can be estimated from Young's modulus of elasticity (E), and paperboard thickness or caliper (c) (Schrier and Verseput, 1967).

$$S = kEc^3$$

Typical results for handsheet bulk and elastic modulus for the four sample genera are given in Table 4. Estimated handsheet stiffness is calculated from these data using this equation for stiffness.

Table 4. Fiber properties for structural papers.

Genus	Bulk cm^3/g	Elastic Modulus km[a]	STFI[b] Compression Strength Nm/g	Estimated Stiffness arbitrary units
Spruce	1.42	830-920	40-43	210-217
W. hem	1.34	900[c]	37-41	180-350
D. Fir	1.67	659-866	31-33	230-371
S. Pine	1.66	888	36	326-348

[a]DeGrace and Page, 1976.   [b]Seth et al., 1986   [c]Horn, 1972

Modulus favors fiber forms giving dense papers, such as the spruces. Caliper invariably favors the coarse fiber species, loblolly pine and Douglas-fir. Since stiffness is linearly dependent on modulus but increases according to the cube of caliper, handsheet stiffness is best for loblolly pine and Douglas-fir. Referring again to Table 3, 90% of the bleached board manufactured in the U.S. is produced in the south using the available southern pines and hardwoods.

The advantage of the coarse southern pine fiber has helped the U.S. paperboard industry for many years, but recent advances with paper machines using several forming sections endangers the southern pine dominance of the paperboard market. Multi-ply paperboard using high modulus fiber furnishes on the outer plies, and a bulking furnish for the inner ply, can improve bending stiffness by 50% over that available with a single furnish paperboard (Fineman, 1985; Engman, 1989). Since spruce and fir refine easily to give high modulus papers for the outer plies, and mechanical pulps and waste paper are good choices for bulking inner plies, the success of the three-ply paper machine offers Canada and the Nordic countries the means to challenge the southern kraft paperboard industry (O'Brien, 1991).

CORRUGATED BOARD

Corrugated board is not really a paper or paperboard but rather an engineered product constructed from paperboard. It is influenced by both the manufacturing process and the nature of the original paperboard products, the linerboard forming the outside of the corrugated sheet, and the medium glued in place between the two liners. Through the years there have been extensive efforts to understand the role of the liners and medium on the performance of the combined board product. The two critical performance tests for linerboard are considered to be compressive modulus and compressive strength (Koning, 1975).

Compressive modulus is generally identical to the modulus of elasticity measured in tensile (Fellers et al., 1980; Wink, et al., 1982) reported in Table 4. Technically, a high elastic modulus requires fibers of low fibril angle (Page et al., 1977) and papers of high density (Page et al., 1979). Modulus is also influenced by the drying restraint applied when producing the paper or handsheet (Setterholm and Chilson, 1965) and can be improved by any means capable of increasing paperboard density, such as improved wet pressing, increased beating (Page et al., 1979) and lower pulp yield (Koning and Haskell, 1979).

Compressive strength is largely a matter of paperboard density (Fellers et al., 1980), but is also influenced by pulp yield (Koning and Haskell, 1979), double-wall thickness and fibril angle (Seth et al., 1986). Under standard pulping and papermaking conditions, species that form higher density papers give higher compressive strength. In Figure 3, compressive strength is plotted against double-wall thickness for the four softwood varieties in the review; spruce gives the best compressive strength, followed by western hemlock, loblolly pine and Douglas-fir (Seth et al., 1986).
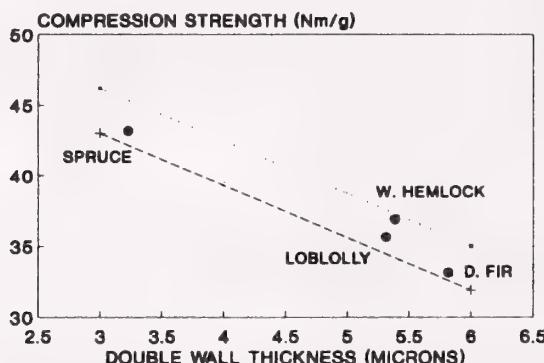
Figure 3. Paperboard compression strength graphed against fiber double wall thickness. Top line is for a 5°, bottom line for a 25° fibril angle (Seth et al., 1986).

In practice, over 80% of the kraft board manufactured in the United States is produced in the south. Compressive strength is largely controlled by sheet density under typical paper machine conditions as seen in Figure 4 (Wink et al., 1982). Southern producers can adjust for the performance characteristics of the southern fibers by improving wet pressing and refining to lower freeness, but it is not possible for the northern producers to adjust for the comparatively high wood costs. For a commodity product such as linerboard, the cost issues are of greater concern than the marginal performance improvement available with thin-walled northern fibers.
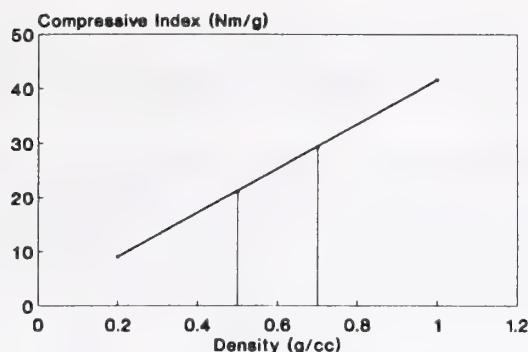
Figure 4. Paper compressive index plotted against paper density. Normal machine densities fall between 0.5 and 0.7 g/cc (Wink et al., 1982).

CONCLUSIONS

Over the past half century, the southern U.S. has come to account for over half of the pulp and paper products produced in the United States. The south provides significant advantages in wood costs but, in most paper grades, the southern pine fiber has poor performance characteristics and requires greater care in papermaking. In mechanical pulps, high energy requirements and poor performance characteristics of the southern pines are a serious problem for the industry, and U.S. expansion in mechanical pulping capacity has been limited since the late 70's. A few southern producers manufacture coated papers, but they must work harder to match the quality of coated paper products from the northeast and north-central states, Canada and northern Europe.

The southern pine industry has benefitted from the improved paper stiffness using the bulky southern pine fibers in production of solid bleached paperboard, but advances in multi-ply papermaking technology have improved the paperboard quality from thin-walled fibers, and northern producers are now able to compete with the southern industry. In corrugated containers, the lower cost of southern fibers dominates the market, but quality would improve with a thinner-walled fiber supply (Fahey and Laundrie, 1968).

Like linerboard, southern pines are a commodity. The paper industry adjusts for their performance limitations to take advantage of the low price and availability. In a quality-conscious world, forest scientists need to search for the means to improve southern pine performance. Low-density southern pine variants with thin fiber walls should give improved performance in most paper products. Since the performance requirements of the paper industry oppose the needs of the solid lumber products industry, the forestry industry needs to develop the capability to select, breed, and deploy trees for a specific end use—if they hope to please both customers.

ACKNOWLEDGMENTS

REFERENCES

Besley,L. 1964. The significance of fiber geometry and distribution in assessing pulpwood quality. TAPPI 47(11):183A.

Bridger,F.A. and Munday,F.D. 1969. The relationship between cartonboard bulge and stiffness. Paper Technology 10(2):109.

Bristow,J.A. and Ekman,H. 1981. Paper properties affecting gravure print quality. TAPPI 64(10):115.

De Grace,J.H. and Page,D.H. 1976. The extensional behavior of commercial softwood bleached kraft pulps. TAPPI 59(7):98.

Engman,C. 1989. Iggesund's experience of CTMP in high-quality packaging board. Pulp and Paper Canada 90(9):57.

Fahey,D.J. and Laundrie,J.F. 1968. Kraft pulps, papers, and linerboard from southern pine thinnings. USDA Forest Service Research Note FPL-182. Forest Products Laboratory, Madison, WI.

Fellers,C., de Ruvo,A., Elfstrom,J. and Htun,M. 1980. Edgewise compression properties: A comparison of handsheets made from pulps of various yields. TAPPI 63(6):109.

Fineman,I. 1985. Let the paper product guide the choice of mechanical pulp. Proceedings International Mechanical Pulping Conference, 1985. p. 203-208.

Grangård,H. 1970. Compression of board cartons, part 1: Correlation between actual tests and empirical equations. Svensk Papperstidning 73(15):462.

Hatton,J.V. and Cook,J. 1990. Managed Douglas-fir forests: IV. Relationships between wood, fibre, pulp and handsheet properties. Proceedings CPPA Annual Mtg. p. A51.

Horn,R.A. 1972. How fiber morphology affects pulp characteristics and properties of paper. Chemicals 26(8):39.

Isenberg,I.H. Revised by Harder,M.L. and Louden,L. 1980. Pulpwoods of the United States, Volume I - Conifers. The Institute of Paper Chemistry, Appleton, WI.

Kartovaara,I., 1989. Mechanical pulp as coating base paper raw material: Strengths, weaknesses and development needs. Preprints, International Mechanical Pulping Conference, Helsinki p. 25-35.

Koch,P. 1972. Utilization of the southern pines. Vol 1., USDA. Forest Service Agriculture Handbook No. 420. U.S. Government Printing Office, Washington, D.C. p. 163-180.

Koning,J.W. 1975. Compressive properties of linerboard as related to corrugated fiberboard containers: A theoretical model. TAPPI 58(12):105.

Koning,J.W. and Haskell,J.H. 1979. Papermaking factors influencing the strength of linerboard weight handsheets. Paperboard Packaging, p. 132.

Kurdin,J.A. 1980. Pulp and paper chemistry and chemical technology. 3rd Ed., Vol. 1, James P. Casey Editor; John Wiley & Sons, NY.

MacLeod,J.M. 1980. Comparing pulp strengths. Pulp and Paper Canada 81(12):128.

Mohlin,U-B. 1989. Fibre bonding ability—A key pulp quality parameter for mechanical pulps used in printing papers. Proceedings,International Mechanical Pulping Conference p. 49.

O'Brien,J. 1991. Temboard invests in its future. Paper Age, Jan.: p. 14-15.

Page,D.H., El-Hosseiny,F., Winkler,K. and Lancaster,A.P.S. 1977. Elastic modulus of single wood pulp fibers. TAPPI 60(4):114.

Page,D.H., Seth,R.S. and De Grace,J.H. 1979. The elastic modulus of paper. TAPPI 62(9):99.

Perry,J.A. 1972. Web offset printing papers. TAPPI 55(5):722.

Schrier,B.H.; Verseput,H.W. 1967. Evaluating the performance of folding cartons. TAPPI 50(3):114.

Seth,R.S., Soszynski,R.M. and Page,D.H. 1986. The effect of wood species on the edgewise compressive strength of paper. TAPPI 69(10):94.

Setterholm,V.C. and Chilson,W.A. 1965. Drying restraint, its effect on the tensile properties of 15 different pulps. TAPPI 48(11):634.

Shallhorn,P. and Karnis,A. 1979. Tear and tensile strength of mechanical pulps. Pulp and Paper Canada 80(12):TR92.

Smith,D.M. 1965. Rapid measurement of tracheid cross-sectional dimensions of conifers. Forest Products Journal (8):325.

Statistics of the Industry. 1990. Lockwood Post directory of the pulp, paper and allied trades, Miller Freeman Publications, Dyer, H. Ed. NY.

Wangaard,F.F. 1969. Cell-wall density of wood with particular reference to the southern pines. Wood Science 1(4):222.

Wink,W.A., Watt,J.A., Whitsitt,W.J. and Baum,G.A. 1982. The effect of fiber axial modulus on compressive strength. Proceedings, 1982 TAPPI Research and Development Conference, p. 37.

Wood handbook: Wood as an engineering material. US Forest Products Laboratory. 1974. U.S. Government Printing Office, Washington, D.C. p. 4-41–4-48.

245

PROGENY TEST DATA SUMMARIZATION PROCEDURES IN THE WESTERN GULF FOREST TREE IMPROVEMENT PROGRAM

W. J. Lowe and J. P. van Buijtenen[1]

Abstract. One of the major objectives of progeny tests in an operational tree improvement program is parental evaluation. To accomplish this objective, data usually needs to be consolidated from progeny tests of different ages, located on different sites, and which contain different variances. Percentage data is subject to scale effects and usually results in a greater weighting of data from younger tests. It also overestimates genetic gain because phenotypic values are used instead of estimates of genetic values. The Western Gulf Forest Tree Improvement Program (WGFTIP) has developed a technique which utilizes standardized performance scores and the coefficient of genetic prediction to estimate parental breeding values at a common age. In addition to reporting an estimated breeding value instead of phenotypic superiority, this technique weights data from older progeny tests heavier than young tests and adjusts for different variances among tests. The incorporation of this technique in the WGFTIP's slash and loblolly pine progeny testing programs is described.

Keywords: Pinus taeda L., Pinus elliottii Engelm., breeding value, indirect selection.

INTRODUCTION

Genetic tests can be established to satisfy numerous objectives (McKinley, 1983); however, parental evaluation and ranking is one of the major functions of genetics tests in operational tree improvement programs. Parental evaluations are used to rogue seed orchards, design new seed orchards, and evaluate advanced generation selections. Standard statistical procedures are available for data analysis in progeny tests that contain parents in a balanced mating design and are replicated across time and space (Lowe et al, 1983). In operational tree improvement programs these conditions are seldom satisfied. Different mating and test designs may be used by cooperators within the same breeding zone. Parents must be evaluated that are established in different genetic tests which are located on different sites and planted in different years. Test precision and measurement age will also vary.

Data summary procedures have been developed to obtain unbiased

[1]Associate Geneticist, Texas Forest Service and Assistant Professor, Department of Forest Science, Texas Agricultural Experiment Station, and Head, Reforestation Department, Texas Forest Service and Professor, Department of Forest Science, Texas Agricultural Experiment Station, College Station, TX

parental evaluations that utilize specific genetic test data as a fixed effect or predicts parental breeding values as a random effect. Hatcher et al (1981) and Cotterill et al (1983) describe various techniques to obtain unbiased estimates of parental performance as a fixed effect. The Best Linear Unbiased Prediction described by White et al (1981) and White and Hodge (1989) is a procedure to estimate breeding values from genetic test data.

The objective of this paper is to describe the procedures developed by the Western Gulf Forest Tree Improvement Program (WGFTIP) to estimate parental breeding values for loblolly pine (Pinus taeda L.) and slash pine (P. elliottii Engelm.) from genetic test data.

MODEL DEVELOPMENT

Progeny tests are routinely measured at young ages (i.e. 5 years); however, parental performance at a mature age needs to be estimated. The general formula for response to indirect selection from Falconer (1981) is:

$$R_m = i \, h_m \, h_j \, \sigma_{pm} \, r_a \qquad (1)$$

Where R_m is mature response, i is selection intensity, h_m is square root of mature heritability, h_j is square root of juvenile heritability, σ_{pm} is mature phenotypic standard deviation and r_a is the genetic correlation between the juvenile and mature trait.

Baradat (1976) defined the coefficient of genetic prediction (CGP) between two traits as:

$$CGP = Cov(A_1A_2) \, / \sigma_{p1}\sigma_{p2} \qquad (2)$$

Where $Cov(A_1A_2)$ is the additive covariance between two traits, and σ_{p1} and σ_{p2} are the phenotypic standard deviations of trait 1 and trait 2, respectively. When the phenotopic mean of trait 1 is shifted one standard deviation, the breeding value of trait 2 is shifted by the CGP multiplied by the phenotypic standard deviation of trait 2. The two traits may be measures of the same variable (i.e. volume) at different ages. In this situation, trait 1 can be a juvenile measurement and trait 2 can be a mature measurement of the same variable.

To use the CGP to calculate response to indirect selection the performance of the juvenile trait must be expressed as standard deviations. The "z" score changes the sample mean to zero and adjusts the variance to one. It is calculated as:

$$z = \frac{(x-\bar{x})}{s} \qquad (3)$$

where x is the observed trait, $\bar{x}$ is the sample mean, and s is the standard deviation.

If equation 2 is substituted into equation 1 and i is replaced by z, the formula for response to indirect selection can be shown as:

$$R_m = z \ CGP \ \sigma_{pm} \qquad\qquad (4)$$

Equation 4 predicts the breeding value of a mature trait (R_m) in response to indirect selection on a juvenile trait when juvenile selection is expressed as a standard deviation from the sample mean and the CGP is estimated between the juvenile and mature traits. Because the z score changes the sample mean to zero, the mean for the mature trait (M_m) needs to be added to the predicted response to estimate the breeding value of the mature trait. This can be expressed as a percent as:

$$PBV_m(\%) = [(M_m + z \ CGP \ \sigma_{pm})/M_m] * 100 \qquad (5)$$

where $PBV_m(\%)$ is predicted breeding value of the mature trait in percent and the other terms are as previously defined.

The model described in equation 5 is valid to predict the breeding value of a mature trait in response to selection on a juvenile trait if each trait is normally distributed. The distribution of most measured traits in forest trees is assumed to be normal. The use of a standard score (z) adjusts for widely differing variances among different progeny tests.

MODEL IMPLEMENTATION IN THE WGFTIP

Members of the WGFTIP have maintained progeny tests for at least 20 years. In many cases, height and diameter measurements were taken at ages 5, 10, 15 and 20 years. An initial review concentrated on all progeny tests that were at least 20 years old. Because very few slash pine tests had reached that age, a second review was made of all slash pine tests that were at least 15 years old. Progeny tests were located in South Arkansas, Louisiana and East Texas. Both open and control-pollinated progeny tests were used in the analysis. Test design varied widely among tests: replications varied from two to twelve and the number of trees per plot ranged from four to one-hundred. If the progeny test had been thinned, the removed volume was added to later measurements to obtain total volume production per acre.

To develop the information needed for equation 5, the data from 31 loblolly pine progeny tests that had reached age 20 were utilized. For slash pine, data from 14 progeny tests that were 15 years old were used. Average progeny test height was used to estimate site index for each test. Site index was used to predict mature volume and the standard deviation among families for volume at age 20 for loblolly pine. Survival or the percent infection by fusiform rust affected the slash pine equations for volume at age 15 and the standard deviation among families for volume production. CGP's were calculated across ages for each test in which significant differences (alpha = 0.1) occurred among

305

genetic entries. Non-significant tests and tests which were not measured on the scheduled interval were not used to estimate CGP's or prediction equations.

Loblolly Pine

In 1983, data from 22 loblolly pine progeny tests that had reached age 20 were used to estimate the parameters required for equation 5. An additional nine progeny tests had reached age 20 by 1986 and the parameters were reestimated using the larger data base. Since no meaningful differences were evident between the parameter estimates for the two data sets, only the results of the last set of analyses will be presented.

Site index (base age 50) was calculated for each progeny test using 20-year mean height by the following equation:

$$Log\ SI = Log(Mean\ Height) - 5.54757\ (1/50 - 1/age)$$

Estimated site indexes at age 20 ranged from 61 to 100 for the 31 progeny tests. Mean progeny tests heights at ages 5, 10 and 15 were used to develop regression equations to predict the site index for each age. Site index was used as the independent variable to predict mean plantation volume at age 20 and the standard deviation among families for volume production. Appendix 1 shows the equations developed for each measurement age.

The coefficient of genetic prediction for volume was calculated between each juvenile age (5, 10 and 15 years) and the mature age (20 years) (Table 1). Approximately 90 percent of the genetic gain in volume growth is obtained by indirect selection for volume at age 10 as compared to direct selection at age 20.

Table 1. Family coefficients of genetic prediction for 20-year volume production with loblolly pine.

Juvenile Age	Mature Age	Coef. of Genetic Pred.
5	20	0.58
10	20	0.63
15	20	0.66
20	20	0.70

Table 2 presents an example of a five-year-old loblolly pine progeny test showing both phenotypic superiorities and estimated breeding values for volume production at age 20. The mean plantation height was 4.45 meters. From the equations in Appendix 1, the site index was estimated to be 88, estimated total volume at age 20 was 16.25

m^3/ha/yr. and the phenotypic standard deviation for volume among families was 2.71 m^3/ha/yr. The volume production of each family was expressed as a standard deviation of the plantation average (z) and the coefficient of genetic prediction was 0.58.

Table 2. Estimated breeding values for volume production at age 20 and percent volume superiorities for a five-year-old loblolly pine progeny test

Parent	Percent Sup.	Breeding Value (%)	Parent	Percent Sup.	Breeding Value (%)
S2PT25	59	80	LBWLob2	101	101
S2PT23	77	89	C25B	104	102
U18A	77	89	CKLOT #2	105	102
C23A	84	93	30Lob1	107	103
U25A	86	93	C21A	114	107
CKLOT #1	86	93	52Lob5	118	108
36Lob8	87	94	S3PT22	122	111
C18B	90	95	T261-2	131	115
S2PT24	92	96	28Lob1	137	118
K-195	94	97			

This procedure can be used for either open or control-pollinated data. For control-pollinated data, the estimated breeding value of each cross is used to determine the general combining ability of each parent in the test. This procedure offers a number of advantages in data summarization. Family ranks within a test are not changed by this procedure and adjustments are made for different site qualities and variances among tests. Because the CGP increases with age, superior growth in older tests receives greater emphasis than in younger tests. Also, reported values are based on estimated breeding values adjusted to a common age instead of phenotypic superiorities at variable measurement ages.

Slash Pine

Fourteen slash pine progeny tests, which were 15 years old, were used in 1989 to develop a similar technique for the prediction of breeding values based on volume at age 15.

Site index (base age 25) was calculated for each progeny test using the 15-year mean test height by the following equation:

LogSI = Log(Mean Height) - 4.767429 (1/25 - 1/age)

Estimated site indices at age 15 ranged from 52 to 68 for the 14 progeny tests. Mean test height at ages 5 and 10 were used to estimate the age 15 site index. Because of mortality caused by fusiform rust, site index alone was not a good predictor of mean test volume at age 15 or the standard deviation among families for volume production. Either average test survival or the percent of trees infected by fusiform rust was added to the model to predict total volume and the phenotypic standard deviation. The equations developed for each measurement age are shown in Appendix 2.

Table 3 presents the coefficients of genetic prediction used in the procedure. Because of rust associated mortality, CGP's were calculated for both rust infection and volume as juvenile selection traits with volume growth at age 15. At age 5, for tests with rust infection rates of 30 percent or greater, the amount of rust infection is a better predictor of age 15 volume than five year volume. This is because rust associated mortality has not affected rapid growing families that are severely infected by fusiform rust. At age 10, volume growth is a better predictor of age 15 volume than rust infection. By this time, mortality has started to affect family rankings for volume.

Table 3. Family coefficients of genetic prediction for 15 year volume production with slash pine.

Juvenile Age	Juvenile Trait	Mature Age	Coef. of Genet. Pred.
5	Rust Infection	15	−0.48
10	Volume	15	0.54
15	Volume	15	0.62

The formulas in Appendix 2 were used to develop the estimated breeding values for volume at age 15 for a slash pine test at age 5 (Table 4). At age 5 the test had a mean height of 3.90 meters, average infection by fusiform rust of 74 percent and 92 percent survival. Site index was estimated as 60, volume at age 15 as 10.26 m^3/ha/yr., and the standard deviation among families as 2.33 m^3/ha/yr. The percent infection by fusiform rust for each family was standardized by the z score. Family K-6 had an above average volume superiority (105 percent) but a below average breeding value because of its high fusiform rust infection rate (91 percent). By age 10, the average survival for K-6 had decreased from 85 to 55 percent because of rust associated mortality. This resulted in the volume superiority dropping to 74 percent.

Table 4. Estimated breeding values for volume production at age 15, percent volume superiority and percent rust infection at age 5 for a slash pine progeny test.

| Parent | Age 5 | | Age 15 | Parent | Age 5 | | Age 15 |
	% Sup.	Rust Infec.	Breed. Value (%)		% Sup.	Rust Infec.	Breed. Value (%)
K-141	52	97	85	OIS-5	64	72	101
K-163	92	92	88	BSS-9	91	72	101
MFCS-1	68	92	88	S5PC1	106	67	104
BSS-6	80	92	89	K-211	104	64	106
K-6	104	91	89	OIS-4	123	58	109
OIS-3	105	87	92	K-179	105	56	111
K-202	97	86	92	BSS-10	143	50	114
K-142	78	84	94	BSS-13	125	41	120
CKLOT	111	82	95	C-103	145	41	120
OIS-1	100	78	97				

This procedure has many of the same characteristics as discussed previously for loblolly pine. However, for it to be effective, fusiform rust infection must be severe enough to detect genetic differences among families. In geographic areas where fusiform rust is not a problem, this procedure is not applicable. In the WGFTIP, rust infection must be greater or equal to 30 percent before the data is used in summarization procedures. Also, the family ranks can change because different traits are used for selection at different ages.

DATA SUMMARIZATION

The procedures previously described are utilized to obtain a breeding value estimate for each parent in a progeny test. Breeding value estimates are averaged across progeny tests to obtain estimated parental evaluation. Open and control-pollinated tests are weighted equally in the final average determined for a clone. For example, a loblolly pine selection that is in two open-pollinated progeny tests with estimated breeding values of 99 and 114 and has general combining ability estimates from three control-pollinated progeny tests of 111, 114 and 121 has an estimated breeding value of 111.8 based on five progeny tests. To be included in the final evaluation each progeny test must have significant differences (alpha = 0.1) among genetic entries. Any test not showing significant differences is deleted prior to averaging parental breeding values across all progeny test. Fusiform rust infection must be equal to or greater than 30 percent in slash pine tests to be used in the data summarization procedure.

SUMMARY

These data summary procedures have been incorporated into the Western Gulf Forest Tree Improvement Program. The equations are easily adapted into an analysis program and the data from each progeny test is expressed as estimated breeding values instead of phenotypic superiorities after analysis. The use of a standardized score adjusts for different variances among tests. Increased growth in older progeny tests receives greater emphasis because the coefficient of genetic prediction increases with age. Data summarization across tests is relatively easy in an incremental procedure. As new test results become available, parental breeding values are calculated for these tests and new averages are computed across all tests. The procedure described above can be developed for any program which has a sufficient data base to develop the required equations.

LITERATURE CITED

Cotterill, P. P., R. L. Correll and R. Boardman. 1983. Methods of estimating the average performance of families across incomplete open-pollinated progeny tests. Silvae Genet. 32(1-2):28-32.

Hatcher, A. V., F. E. Bridgwater and R. J. Weir. 1981. Performance level-standardized score for progeny test performance. Silvae Genet. 30(1-6):184-7.

Lowe, W. J., R. Stonecypher, and A. V. Hatcher. 1983. Progeny test data handling and analysis: In Progeny Testing of Forested Trees. S. Coop. Series Bull. No. 275. p. 51-67.

McKinley, C. R. 1983. Objectives of progeny tests: In Progeny Testing of Forest Trees. S. Coop. Series Bull., No. 275. p. 2-5.

White, T. L., G. R. Hodge and M. A. Delorenzo. 1987. Best linear prediction of breeding values in forest tree improvement: In Statistical Considerations in Genetic Testing of Forest Trees. S. Coop. Series Bull. No. 324. p. 99-121.

White, T. L. and G. R. Hodge. 1989. Predicting Breeding Values with Applications in Forest Tree Improvement. Kluwer Academic Publishers, Norwell, MA. 367 pp.

Appendix 1. Equations to estimate loblolly pine breeding values for volume at age 20.

A. Site Index (SI)

Age 5: $SI = 41.855 + 10.276 \text{ (Mean Height)}$
$R^2 = 0.64$

Age 10: $SI = 13.962 + 6.976 \text{ (Mean Height)}$
$R^2 = 0.87$

Age 15: $SI = 6.041 \text{ (Mean Height)}$
$R^2 = 0.83$

Age 20: $SI = 4.813 \text{ (Mean Height)}$
$R^2 = 0.99$

B. Total Volume (Age 20)

$Volume = -7.034 + 0.266 \ SI$
$R^2 = 0.58$

C. Standard Deviation Among Families (Age 20)

$Std. \ Dev. = 0.031 \ SI$
$R^2 = 0.23$

Appendix 2. Equations to estimate slash pine breeding values for
             volume at age 15.

A. Age 5

    Site Index (SI) = 37.695 + 5.805 (Mean Height)
                $R^2 = 0.81$

    Volume (Age 15) = -12.780 + 0.225 SI + 0.103 (Mean Survival)
                $R^2 = 0.74$

    Std. Dev. Among Families (Age 15) = -6.777 + 0.151 SI
                                    $R^2 = 0.71$

B. Age 10

    Site Index (SI) = 26.886 + 3.624 (Mean Height)
                $R^2 = 0.64$

    Volume (Age 15) = -15.043 + 0.277 SI + 0.102 (Mean Survival)
                $R^2 = 0.82$

    Std. Dev. Among Families (Age 15) = -9.883 + 0.2317 SI
                                        -0.027 (Mean Infection)
                                    $R^2 = 0.83$

C. Age 15

    Site Index (SI) = 0.930 + 4.334 (Mean Height)
                $R^2 = 0.99$

    Volume (Age 15) = -17.924 + 0.337 SI + 0.108 (Mean Survival)
                $R^2 = 0.88$

    Std. Dev. Among Families (Age 15) = -9.8836 + 0.232 SI
                                        -0.027 (Mean Infection at
                                                Age 10)
                                    $R^2 = 0.83$

POSTER ABSTRACTS

GENETIC IMPROVEMENT OF Pinus radiata COMBINED WITH INTEGRATED NURSERY AND OUTPLANTING SYSTEMS LOWER PLANTATION ESTABLISHMENT COSTS.

Roger J. Arnold.[1]

Key Words: Tree improvement, bare-root seedling, seedling quality, nursery practice, plantation establishment.

Abstract. Significant genetic improvement in Pinus radiata for tree form and for the consequent proportion of trees acceptable as crop elements has been achieved over the past decade in New Zealand. Concurrent improvement has occurred in bare-root seedling outplanting systems. Together, genetic and outplanting improvements have enabled initial operational stocking levels to be reduced to as low 800 trees per hectare. Short term commercial benefits of the combined improvements include 20 % reduction in establishment costs per hectare, despite higher planting and stock costs per tree. Long term benefits identified include final crop volume gains.

[1] Graduate student. School of Forestry, N.C. State University, P.O. Box 8002, N.C.S.U., Raleigh, N.C. 27695.

CLONE INFLUENCE ON AGROBACTERIUM-MEDIATED DNA TRANSFER TO PINUS RADIATA

Ben A. Bergmann and A.-M. Stomp
Forestry Department, North Carolina State University

Research in the last five years has shown that the host range of *Agrobacterium tumefaciens* extends to many species in the genus *Pinus* (1). This observation provides the basis for developing DNA transfer methods in pine species using *Agrobacterium* as the gene vector. Successful DNA transfer depends on a specific host-pathogen interaction involving genes of both the *Agrobacterium* strain (1) and the host plant (2). In crop plants where sufficient breeding has occurred to produce inbred lines or cultivars, the genetics of the host-pathogen relationship can be explored using cultivar seedlings. In pines, cultivars or inbred lines do not exist. Therefore, we have examined the influence of host genotype on *Agrobacterium*-mediated DNA transfer in *Pinus radiata* using clones produced by tissue culture.

In this study, we compared the frequencies of gall formation in clonal *P. radiata* shoots produced by the method of Aitken-Christie (3). Shoots of identical clonal lines or a clonal mix were either inoculated *in vitro* or after establishment in the greenhouse. Seedlings of the same seed lot used to produce the tissue culture clonal plantlets were grown and inoculated at the same time as the clonal plants. *Agrobacterium* virulent strains C2/74, wild type 542, wild type 542 carrying pEND4K (confering kanamycin resistance), and the avirulent strain A136 were used for inoculation. Inoculation consisted of stabbing the shoot or stem several times close to the apex with a scalpel blade dipped in freshly grown *Agrobacterium* cultures. Wound-only controls were maintained to compare the hypertrophy around the wound area with and without the presence of bacteria. As verification of foreign gene transfer, *in vitro* galls (inoculated with 542/pEND4K) were placed onto medium containing kanamycin (resistance from the npt-II gene), and galls produced *ex vitro* were analysed for agropine/mannopine synthesis.

Frequency of gall formation differed significantly among clones *in vitro* and ranged from 0 and 39%. Strains 542 and C2/74 did not differ in their ability to induce galls in clonal plantlets inoculated *in vitro*. Although clones varied significantly in the number of shoots produced per original embryo and shoot rootability, no correlation was found between these parameters and the frequency of gall formation *in vitro*. Frequency of gall formation differed significantly among clones inoculated under greenhouse conditions and ranged from 26 to 41%. Gall formation frequency on greenhouse clonal trees was positively correlated with growth rate. Across all clones, gall formation was lower in clonal shoots inoculated *in vitro* (24%) compared to trees inoculated in the greenhouse (38%). Rankings of clones by the frequency of gall formation were different when comparing *in vitro* inoculation to greenhouse inoculation. No difference in the frequency of gall formation was observed between seedlings and a 21-clone mixed population of trees produced through tissue culture.

The major finding of this work is that clonal genotype does significantly influence transfer of foreign DNA via direct stem inoculation with *Agrobacterium*, but differing cultural conditions (*in vitro* versus greenhouse) and tree growth rates are of at least equal importance.

[1]Stomp, A-M *et al*. 1990. Pl Physiol 92:1226-1232.
[2]Hinchee, MAW *et al*. 1988. Bio/technology 6:915-922.
[3]Aitken-Christie, J *et al*. 1988. In: Genetic Manipulation of Woody Plants. Plenum Press, New York: 413-432.

TRANSFORMATION OF *LIQUIDAMBER STYRACIFLUA* L. (SWEETGUM) USING *AGROBACTERIUM TUMEFACIENS*

Z.-Z. Chen and A.-M. Stomp
Forestry Dept., North Carolina State University
Raleigh, NC

We have produced the first transgenic sweetgum plants using *Agrobacterium*-mediated DNA transfer. Transformation was accomplished by co-cultivation of leaf pieces or nodules with a disarmed binary *Agrobacterium tumefaciens* (pBI121 in C58z707). The C58 background was used because wild type C58 gave good gall formation on sweetgum seedlings. The mini-plasmid (pBI121) contains the selectable marker, neomycin phosphotransferase (NPT II) under the NOS promoter and the unselectable marker, beta-glucuronidase (GUS) under the CaMV35s promoter. Co-cultivated tissue was transferred to 500 mg/L, each, of carbenecillin and cefotaxime to decontaminate the tissue and 40 mg/L kanamycin sulfate to select transformed cells. Tissue was carried under selection for 6 months, subcultured every 2 weeks, in liquid medium to proliferate nodules and segregate a transgenic nodule population. Cultures were checked periodically for GUS expression during selection. After cultures grew freely without carbenecillin and cefotaxime, indicating decontamination of the tissue, shoot formation was induced on agar medium containing kanamycin following the procedures developed for nodule cultures of sweetgum (Chen & Stomp, in accompanying abstract). Individual shoots were excised from shoot producing nodule masses and rooted on medium containing kanamycin. Transformation has been verified using an ELISA test for the presence of NPT II, positive GUS staining and quatitative assay and analysis of DNA isolated from GUS positive plants by Southern hybridization. A number of transgenic nodular callus lines and transgenic plants have been produced using this method. These procedures provide a routine method for the production of transgenic sweetgum plants or cell cultures.

ORGANOGENESIS FROM NODULAR CULTURES OF *LIQUIDAMBER STYRACIFLUA* L. (SWEETGUM)

Z.-Z. Chen and A.-M. Stomp
Forestry Dept., North Carolina State University
Raleigh, North Carolina

Plantlet regeneration has been obtained from nodule cultures of *Liquidamber styraciflua* L. (sweetgum). Nodules in a continuum of sizes were produced from leaf pieces by culture in liquid Woody Plant Medium (WPM) (Lloyd & McCown 1981) containing 5 uM benzyladenine (BA) and 0.5 uM naphthaleneacetic acid (NAA). Three sizes of nodules were evaluated for growth and regenerative capacity. The best culture conditions for shoot induction were observed using nodules with a diameter 2.5 mm cultured on WPM containing 5 uM BA and no auxin. Root induction alone was induced from all nodule sizes in liquid medium containing 0 - 0.5 uM BA and 0 - 5 uM NAA. We have taken this system and produced transgenic sweetgum plants using *Agrobacterium* (see accompanying abstract).

We have identified three distinct morphogenic stages occurring during nodule development: 1) vascularization, 2) shoot initiation, and 3) root formation. The smallest nodules have no vascularization and consists of thin walled cells. These small nodules differentiate vascular elements (tracheids) in the center of the nodule when it is still quite small (less than 2mm). The appearance of vascular elements precedes shoot or root formation. The ability of this system to specifically produce tracheids, shoot or roots in response to culture conditions provides a useful system for the study of factors regulating vascular, root and shoot development in woody plants.

Lloyd, DG and BH McCown (1981) Proc. Int. Plant Prop. Soc. 30:421-427.

Rejuvenation of Loblolly Pine Vegetative Buds
by
Shoot-tip Micrografting Techniques.

Barbara S. Crane

Successful vegetative propagation of some mature conifer species depends on the ability to rejuvenate mature tissue and induce it to form juvenile tissue. One treatment often used for the induction of juvenility involves grafting of mature buds onto young rootstocks.

Micrografting techniques were tested as a means of rejuvenating meristematic tissue of 8-year-old loblolly pine (Pinus taeda L.) vegetative buds. In the main experiments, apical scions (the apical dome plus some sub-apex tissue, measuring 0.1-0.3 mm) were grafted onto 4-, 8-, 12-, and 16-week-old loblolly pine and slash pine (P. elliottii Engelm. var. elliottii) seedlings. They were grown singly in tubes under incandescent, fluorescent and "gro-sho" lights, creating individual greenhouse environments. Cleft, veneer and budding graft cuts were employed on foliated and defoliated seedlings. Both the epicotyl and hypocotyl regions served as graft sites. Seedlings were grafted in fall, winter, spring, and summer. In supplemental experiments, three chemicals (sodiumdiethyldithiocarbamate, polyvinylpyrrolidone, and benzoadeninepurine, at two concentrations each) and activated charcoal were applied to graft sites in attempt to improve grafting success and to induce juvenility. Rootstocks grafted in the fall were maintained in the light following grafting. Rootstocks grafted in subsequent experiments were maintained in the dark for five days following grafting, then placed in the light.

To assess anticipated successful rejuvenation, primary and mature loblolly pine needles were cultured *in vitro* on modified basal media (DCR). Primary needles showed abundant callusing, mature needles died. Additionally, apical scions from mature buds were grafted on primary needle callus to determine meristem tissue viability and activity. All scions responded by either callusing or by swelling, elongating and turning a bright green. The apical scions were therefore viable and capable of callusing and growing at the time of grafting.

There were no successful graft takes with 962 attempts. Although rootstocks developed healthy callus at the graft site, the scions did not. Scions became necrotic and died within 3-15 days, depending on treatment. Histological examination of the graft union tissue revealed a barrier of necrotic cells between the rootstock and scion, resin deposition, no vascular connections, nor cambial joining between the graft components.

It would appear that the micrografting methods employed in this study did not provide favorable circumstances for apical scions to fuse with rootstocks, or conversely. In summary, micrografting may not be a viable alternative in attempting rejuvenation and propagation of mature loblolly pine vegetative buds.

INITIATION OF ADVENTITIOUS SHOOTS
FROM VITREOUS PINUS PALUSTRIS MICROPROPAGULES

Alex M. Diner
USDA Forest Service
Southern Forest Experiment Station
Alabama A&M University
P.O. Box 1208, Normal, AL 35762

Pinus palustris micropropagules of normal phenotype were generated from vitreous buds and shoots otherwise inappropriate for further use. Twenty-three vitreous shoots which had elongated to approximately 1 cm were cut to needles and stem segments, while younger buds in 25 collective vitreous clusters were pried/broken apart from each other. Tissues were then applied to a 0.8% agar-solidified Brown and Laurence mineral salts medium containing 44 uM 6-benzyladenine for 14 days. Following transfer to a growth regulator-free medium containing 1% activated charcoal, new bud growth was identified within 2 weeks.All buds initiated and elongated from vitreous shoots were normal in appearance(av.=3). With 1 exception, buds initiated from fragments of vitreous bud clusters (av.=2/0.5 cc) appeared normal. Three buds initiated from one vitreous cluster were themselves vitreous. All newly initiated buds appeared to develop from bases of shoot needles or apical regions of isolated vitreous buds. This procedure may permit rescue and remultiplication of vitreous, select genotypes for subsequent, functional micropropagation.

SIMULATION MODEL FOR SLASH PINE SEED ORCHARDS

C. W. Fatzinger
USDA Forest Service
Southeastern Forest Experiment Station
Olustee, Florida

W. N. Dixon
Florida Department of Agriculture & Consumer Services
Division of Forestry
Gainesville, Florida

Faced with the task of formulating pest management programs, most orchard managers rely on general theories that may not actually provide the answers for problems unique to their seed orchards. One management tool--a simulation model--is now available. We developed the model for slash pine seed orchards to estimate gains or losses likely to result from specific pest management strategies.

The model runs on IBM-compatible personal computers. It employs self-installation and configuration procedures for the convenience of the user. The model is menu-driven for ease of use. Information and reports appear on screen. Printed reports are also available.

The model can use specific orchard survey data. It contains default values for damage incurred by female strobili and seed factors and for the efficacies of different pesticide treatments. Any of these default values can be changed to conform with expected values for a particular orchard. The user can rapidly compare the results of two or more pest management strategies.

ADVENTITIOUS SHOOT FORMATION INDUCED IN *PINUS TAEDA* L. (LOBLOLLY PINE) COTYLEDONS BY THIDIAZURON

C. G. Giles and A.-M. Stomp
Forestry Dept., North Carolina State University
Raleigh, NC

The cotton defoliant thidiazuron has been used to stimulate shoot proliferation *in vitro* from both Malus (van Nieuwkerk, Zimmerman & Fordham 1986) and Acer (Kerns & Meyer 1986) shoot elongation. A known cytokinin response in gymnosperms is the production of adventitious shoots from embryos or embryo organs. Mott and Amerson (1982) have developed such a procedure using cotyledons from 5-day germinated loblolly pine seeds and benzyladenine as the cytokinin. The experiment described below compared thidiazuron's (TDZ) ability to stimulate adventitious shoot formation from loblolly pine cotyledons relative to that of benzyladenine (BA). A concentration gradient was set up using TDZ and BA to find the optimal concentration for producing maximal amounts of shoots. Basal medium was formulated following the procedures of Mott and Amerson (1982). Control cotyledons were incubated on media without plant growth regulators or with 0.05 uM NAA. All media containing BA or TDZ contained 0.05 uM NAA. Thidiazuron and BA were tested at 1, 10, 20, 40, 60 and 100 uM concentrations. Cotyledons from 18 embryos were incubated on each of the 8 treatments for each replicate. The experiment was replicated 3 times. Final data were taken at the end of 17 weeks. Total number of shoots greater than 5 mm per clone for each treatment/concentration combination was included in the data set.

Cotyledons on all thidiazuron concentrations except 1 uM produced adventitious shoots. Based upon frequency of shoot-producing clones and the mean number of shoots produced per clone, 40 uM BA and 60 uM TDZ were the optimal concentrations. The observed trends in the number of shoots produced by shoot-forming clones were similar for BA and TDZ, with TDZ values being consistently lower than those for BA. Shoots produced by thidiazuron were identical in appearance to those produced by benzyladenine.

Kerns HR and MM Meyer, Jr. (1986) Hortscience 21:1209-1210.

Mott RL and HV Amerson (1982) N. C. Agric. Res. Ser. Tech Bull. #271 1-14.

van Nieuwkerk JP, Zimmerman RH and I Fordham (1986)
Hortscience 21:516-518.

ORGANIZATION AND VARIATION OF rDNA IN PINUS SYLVESTRIS

Päivi Karvonen, Matti Karjalainen and Outi Muona
Department of Genetics, University of Oulu
Oulu, Finland

We examine the chromosomal organization, structure and variation of ribosomal RNA genes (rDNA) in Scots pine (Pinus sylvestris). In a conifer genome, rRNA genes are present in thousands of copies, which are tandemly arranged at several loci. rRNA-loci are also called nucleolus organizers (NORs) because of their ability to form nucleoli in interphase cells. NOR-specific silver staining was used to reveal the number and location of rDNA-containing sites in Scots pine chromosomes. There are at least 8-10 NORs in the haploid Scots pine genome (n=12), distributed on most chromosomes.

The structure and variation of rDNA repeats were studied by Southern hybridizations using nonradioactive digoxigenin labeling and detection techniques. The size of the rDNA repeat unit proved to be about 27 kb. This corresponds to the size of the rDNA unit in Pinus radiata (Cullis et al.). A restriction map of the coding region has been constructed.

rDNA variation was studied with 11 restriction endonucleases in Scots pine populations from southern (61°N) and northern (67.5°N) Finland. A heterologous probe (the complete rDNA repeat unit from flax) was useful in detecting restriction site variation both within and between populations. However, no rDNA repeat length heterogeneity was detected in this study. Some of the rDNA variants detected with Hind III seem to be present only in the northern Scots pine population, but only small samples have been studied by now. In order to characterize the structure and variation of rDNA more thoroughly, cloning of the rDNA repeat unit of Scots pine has been undertaken. This will facilitate the detection of variability in the intergenic spacer (IGS) region of rDNA.

SOMATIC EMBRYOGENESIS IN TISSUE CULTURES OF AMERICAN CHESTNUT

S.A. Merkle, W.J. Lott, B.A. Watson-Pauley, A.T. Wiecko and D.T. Carraway
School of Forest Resources, University of Georgia, Athens, GA 30602

Cultures were initiated from developing ovules and excised embryos of American chestnut [*Castanea dentata* (Marsh.) Borkh.], collected from 5 source trees on three dates during early and middle stages of embryo development. Ovule and embryo explants were cultured initially on semisolid induction medium containing 0.25 mg BA/l and either 6 mg NAA/l or 4 mg 2,4-D/l, for 1 or 2 weeks. Cultures were then transferred to either hormone-free medium or medium with 0.25 mg BA/l, or were maintained on the original induction media. Ovules collected approximately 6 weeks postanthesis from 3 of the 5 trees produced embryogenic cultures. Those pulsed for 1 or 2 weeks on auxin-containing media and subsequently transferred to media without auxin produced multiple embryos directly from the radicle end of the zygotic embryo. Cultures maintained on auxin-supplemented media initially produced proembryogenic masses, which formed globular and heart-stage embryos as they aged. Transfer of clusters of somatic embryos from auxin-supplemented media to hormone-free medium promoted maturation of embryos to the cotyledon-stage.

MICROPROJECTILE MEDIATED STABLE TRANSFORMATION IN *PINUS TAEDA* L. (LOBLOLLY PINE)

A.-M. Stomp, D. Robertson and J. Parsons
Forestry Dept., North Carolina State University
Raleigh, NC

Methods for stable gene transfer and expression are critical for the advancement of molecular biology in forest tree species. We report the first stable expression of neomycin phosphotransferase in meristematic tissue of loblolly pine. Approximately 4000 loblolly pine cotyledons were bombarded with tungsten microprojectiles carrying pRT99 plasmid DNA, using the DuPont PD-1000 biolistic particle delivery system optimized for transient expression (GUS) with this tissue. Plasmid pRT99 carries neomycin phosphotransferase (NPT II) and beta-glucuronidase (GUS), each under the control of a CaMV35s promoter. Cotyledons were cultured for shoot production following the procedures of Mott and Amerson (1982), with the medium containing either 10 or 20 mg/L kanamycin sulfate. Cotyledons were subcultured every two weeks. Gene expression was followed by growth and meristematic development on kanamycin and by GUS histochemical staining. After 3 months in culture, approximately 200 cotyledons remained alive and had proliferated meristematic tissue and 14 shoots. Meristematic tissue has been analyzed for GUS activity, the presence of NPT II protein (by ELISA), and has been found to contain NPT II protein, but assays of GUS expression levels are inconclusive. We are in the process of analyzing DNA from this material and of rooting shoots which continue to grow on kanamycin.

Mott, RL and HV Amerson (1982) N. C. Agric. Res. Ser. Tech. Bull. #271. 1-14.

Phenylalanine Ammonia-Lyase in Loblolly Pine
R. W. Whetten and R. R. Sederoff
Department of Forestry, North Carolina State University
Raleigh, North Carolina

L-phenylalanine ammonia-lyase (PAL) is the first enzyme in the phenylpropanoid biosynthetic pathway of plant secondary metabolism. Products of this pathway in conifers include flavonoid pigments in foliage and stilbenes, tannins, polyphenols and lignin in wood. Lignin is the most abundant phenylpropanoid compound in conifers, and makes up about 30% of the dry weight of loblolly pine wood. Biotechnological manipulation of the phenylpropanoid pathway could change lignin content and yield wood with different properties. We have purified PAL from developing wood of loblolly pine, raised antibodies against the purified protein and isolated a partial cDNA clone of PAL. No evidence of heterogeneity has been been detected in pine PAL at the protein level, in contrast to reports of multiple isozymes of PAL in herbaceous angiosperms. PAL appears to be encoded by a single gene in the pine genome, based on Southern blot analysis of genomic DNA using the pine PAL cDNA as a probe.
We are now working to isolate genomic clones of the pine PAL gene and its associated regulatory elements. We plan to investigate the mechanisms that control levels of PAL activity in developing wood, to lay the foundation for later attempts to manipulate PAL during development.

EXPRESSION OF FOREIGN GENES IN TRANSGENIC YELLOW-POPLAR PLANTS

H.D. Wilde[1], R.B. Meagher[2], and S.A. Merkle[1]

[1]School of Forest Resources and [2]Department of Genetics
University of Georgia, Athens, GA 30602

Genetic improvement of yellow-poplar (*Liriodendron tulipifera*) by traditional breeding strategies has been a slow process because, like all forest trees, it has a long generation time. Genetic engineering by recombinant DNA technology has the potential to accelerate the introduction of new traits into forest trees. Certain considerations, however, should be taken into account when designing strategies to produce transgenic trees: (1) few tree species currently can be regenerated from protoplasts, (2) chimeras cannot be easily eliminated, as with herbaceous plants, by sexual transmission of the transformed genotype, and (3) large-scale production of transgenic trees will be accomplished most efficiently by somatic embryogenesis.

Transformation by microprojectile bombarbment offers an approach to the production of transgenic plants from the growing number of tree species that can be regenerated from tissue culture. In this study, plasmid DNA was introduced by a particle gun into single cells and small cell clusters isolated from an embryogenic suspension culture of yellow-poplar. The plasmid pBI 121 carried marker genes encoding ß-glucuronidase (GUS) and neomycin phosphotransferase (NPT II). Under constant antibiotic selection, transformed calli were isolated, subcultured into liquid medium, and regenerated into plants by somatic embryogenesis.

The number of copies of the GUS gene in independently transformed callus lines ranged from 3 to 30. An ELISA for NPT II and a fluorometric assay for GUS showed that the expression for both enzymes varied by less than four-fold among callus lines. A histochemical assay for GUS revealed a heterogeneous pattern of staining with the substrate X-gluc. However, expression of GUS and NPT II was detected by quantitative assays both in cell clusters that reacted positively (blue) and negatively (white) with X-gluc. Somatic embryos induced from transformed cell cultures were found to be uniformly GUS-positive by histochemical analysis. All transgenic plants sampled expressed the two marker genes in both root and shoot tissues. GUS activity was found to be higher in leaves than roots by fluorometric assays. Conversely, roots expressed higher levels of NPT II than leaves.

LIST OF ATTENDEES

Adams, John C.
Louisiana Tech. University
Route 6, Box 1240
Ruston, LA 71270
(318) 257-4724

Aimers-Halliday, Jacqui
Texas A&M University
Dept. of Forest Science
College Station, TX 77843-2135
(409) 845-5033

Allen, Robert M.
Clemson University
Dept. of Forest Resources
271 Lehotsky Hall
Clemson, SC 29634

Arnold, Roger J.
North Carolina State University
P.O. Box 5032
Raleigh, NC 27650
(919) 737-7581

Ashley, Roy C.
State of Tennessee
Division of Forestry
701 Broadway
Nashville, TN 37243-0444
(615) 742-6616

Barbour, Jill
National Tree Seed Laboratory
Route 1, Box 182 B
Dry Branch, GA 31020
(912) 744-3313

Barnett, James P.
USDA Forest Service
Southern Forest Experiment Station
P.O. Box 5500
Pineville, LA 71360
(318) 473-7216

Beaver, Glen
USDA Forest Service
201 Woodland Drive
Murphy, NC 28906
(704) 837-5152

Bergmann, Ben A.
North Carolina State University
Box 8002, Dept of Forestry
Raleigh, NC 27695-8002
(919) 737-7573

Berrang, Paul C.
USDA Forest Service
Center for Forest
  Environmental Studies
Dy Branch, GA 31020
(912) 744-0212

Blush, Tom
Westvaco Corporation
Forest Science Laboratory
P.O. Box 1950
Summerville, SC 29484
(803) 871-5000

Bosch, Carol O.
The Bosch Nursery, Inc.
Route 2, Box 142A
Jonesboro, LA 71251
(318) 259-9484

Bosch, Dorothy S.
The Bosch Nursery, Inc.
Route 2, Box 142A
Jonesboro, LA 71251
(318) 259-9484

Bosch, Leonard W.
The Bosch Nursery, Inc.
Route 2, Box 142A
Jonesboro, LA 71251
(318) 259-9484

Bosch, Ted W.
The Bosch Nursery, Inc.
Route 2, Box 142A
Jonesboro, LA 71251
(318) 259-9484

Bower, Ralph
MacMillian Bloedel, Inc.
P.O. Box 336
Pine Hill, AL 36769
(205) 682-9882

Bradshaw, Toby
University of Washington
Dept. of Biochemistry SJ-70
Seattle, WA 98195
(206) 543-4662

Bramlett, David L.
USDA Forest Service
Route 1, Box 182A
Dry Branch, GA 31020
(912) 744-0261

Brantley, Franklin
Weyerhaeuser Company
Route 1, Box 119 A
Lyons, GA 30436
(912) 526-8612

Bridgwater, Floyd
USDA Forest Service
North Carolina State University
Forestry Dept.
P.O. Box 8002
Raleigh, NC 27695-8002
(919) 737-3168

Brown, George F., Jr.
Alabama A&M University
Dept. of Plant and Soil Science
P.O. Box 1208
Normal, AL 35762
(205) 851-5462

Burris, Leon
Weyerhaeuser Company
P.O. Box 1060
Hot Springs, AR 71902
(501) 624-8530

Caldwell, Tom
International Forest Seed Company
P.O. Box 490
Odenville, AL 35120
(800) 633-4506

Campbell, Ronald D.
International Paper Company
P.O. Box 915
Springhill, LA 71075
(318) 994-2546

Carraway, Daniel T.
University of Georgia
School of Forest Resources
P.O. Box 2298
Athens, GA 30612
(404) 542-6515

Charomaini, Mohammad
North Carolina State University
School of Forest Resources
2718 Clark Ave, Room 201
Raleigh, NC 27607
(919) 839-0062

Chase, Charles D.
St. Joseph Land
  and Development Company
Route 1, Box 70
Lamont, FL 32336
(904) 997-0526

Coleman, Stephen W.
Boise Cascade
P.O. Box 45
Singer, LA 70660
(318) 463-9681

Cox, Russell A.
Tennessee Division of Forestry
P.O. Box 2666
Knoxville, TN 37901
(615) 594-6432

Crane, Barbara S.
Westvaco Corporation
Forest Science Laboratory
P.O. Box 1950
Summerville, SC 29484
(803) 871-5000

Delaney, Derwood
Louisiana Forest Seed Company, Inc.
303 Forestry Road
Lecompte, LA 71346
(318) 443-5026

Delaney, John
Louisiana Forest Seed Company, Inc.
303 Forestry Road
Lecompte, LA 71346
(318) 443-5026

DeWald, Laura E.
Warren Wilson College
P.O. Box 5025
701 Warren Wilson Road
Swannanoa, NC 28778
(704) 298-3325

Dhyani, Shiv Kumar
Mississippi State University
P.O. Drawer FR
Mississippi State, MS 39762
(601) 325-2946

Diner, Alex M.
USDA Forest Service
Southern Experiment Station
P. O. Box 1208
Normal, AL
(205) 851-5462

Dinus, Ronald J.
Institute of Paper Science
  and Technology
575 14th Street NW
Atlanta, GA 30318
(404) 853-9565

Dix, Steve G.
USDA Forest Service
HC. 69
Box 1532
Moncks Corner, SC 29461
(803) 336-3248

Douglass, Susan
Weyerhaeuser Company
P.O. Box 1060
Hot Springs, AR 71902
(501) 624-8507

El-Kassaby, Y. A.
Canadian Pacific Forest
  Products Limited
Tahsio Pacific Region
Saavnich Forestry Centre
Saanichton, B.C.
CANADA VOS 1MO

Farnum, Peter
Weyerhaeuser Company
WTC 1A3
Tacoma, WA 98477
(206) 924-6318

Fatzinger, Carl W.
USDA Forest Service
Southeastern Forest
  Experiment Station
Forestry Sciences Laboratory,
P.O. Box 70
Olustee, FL 32072
(904) 752-0331

Feret, Peter
Virginia Polytechnic Institute
Department of Forestry
228 Cheatham Hall
Blacksburg, VA 24061
(703) 961-5943

Ford-Logan, Jane
USDA Forest Service
Southern Forest Experiment Station
P.O. Box 1208
Normal, AL 35762
(205) 851-5462

Foster, Larry
International Paper Company
1005 River Road
Selma, AL 36701
(205) 875-8306

Gates, James
USDA Forest Service
2500 Shreveport Highway
Pineville, LA 71360
(318) 473-7195

Gerwig, Davis M.
Westvaco Corporation
P.O. Box 1950
Summerville, SC 29484
(803) 556-8391

Gladstone, William T.
Southeastern Journal
  of Applied Forestry
Route 2, Box 140AA
Avoca, NY 14809
(607) 566-9266

Greenwood, Michael S.
University of Maine
122 Nutting Hall
Orono, ME   04469
(207) 581-2838

Gresham, Homer H.
Champion International
P.O. Box 549
Jay, FL   32565
(904) 675-4536

Groover, Andrew
University of Georgia
School of Forest Resources
295 Sycamore Drive #1
Athens, GA   30605
(404)   542-6515

Guinness, William
Bowater Inc.
Carolina Division Wooodlands
P.O. Box 7
Catawba, SC   29704
(803)   329-6653

Hannah, Wayne
Route 3, Box 20-B
Kirbyville, TX   75956
(409) 423-5228

Hatcher, Alice
North Carolina State University
Box 8002
Raleigh, NC   27695-8002
(919)   737-3168

Heard, Jimmy
Willamette Industries, Inc.
General Delivery
Taylor, LA   71080
(318)   377-4465

Heaton, Louis C.
Louisiana Office of Forestry
P.O. Box 1628
Baton Rouge, LA   70821
(504)   925-4500

Hendrickson, John A.
Scott Paper Company
P.O. Box 899
Saraland, AL   36571
(205) 675-2932

Hiatt, Evelyn
Clemson University
Dept. of Forest Resources
271 Lehotsky Hall
Clemson, SC   29634

Hicks, Van, Jr.
Louisiana Office of Forestry
P.O. Box 837
Deridder, LA   70634
(318)   463-5509

Hodge, Gary
University of Flordia
118 Newins-Ziegler Hall
0303 IFAS
Gainesville, FL   32611-0303
(904)   376-2197

Hodges, James
Champion International Corporation
Western Carolina Seed Orchard
P.O. Box 100
Silverstreet, SC   29145
(803)   995-4842

Holifield, Quintaniay
Alabama A&M University
6126 Valley Park Drive, NW
Huntsville, AL 35810
(205)   859-2026

Huber, Dudley A.
University of Florida
118 Newins-Ziegler Hall
Gainesville, FL   32611
(904)   392-1850

Isaacs, William J.
SouthPine, Inc.
P.O. Box 530127
Birmingham, AL   35253
(205) 879-1099

Jahromi, Siroos T.
International Paper Company
Route 1, Box 421
Bainbridge, GA   31717
(912)   246-3642

Jett, J. B.
North Carolina State University
Box 8002
Raleigh, NC 27695-8002
(919) 737-3168

Johns, Bill
USDA Forest Service
Route 1, Box 73
Bentley, LA 714 7
(318) 765-3554

Joyce, Dennis
Ontario Ministry of Natural Resources
258 Queen Street, East
Sault Ste. Marie, Ontario
CANADA PGA 5N5
(705) 945-6720

Kang, Hyun
USDA Forest Service
North Central Forest
  Experiment Station
1630 Linden Drive
Madison, WI 53706
(608) 262-1669

Kitchens, Frank
ITT Rayonier Inc.
P.O. Box 819
Yulee, FL 32097
(904) 225-5393

Kolnik, Timothy J.
USDA Forest Service
Ocoee Ranger District
Route 1 Box 348-D
Benton, TN 37307
(615) 338-5201

Kormanik, Paul P.
USDA Forest Service
Southeastern Forest
 Experiment Station
Forestry Sciences Laboratory
Carlton/Green Streets
Athens, GA 30602
(404) 546-2435

Kremer, Antoine
INRA
Laboratorire de genetique et d'ame
  lioration des arbres forestiers
Pierroton, 33610-Cestas
FRANCE
33 56 680303

Kriebel, Howard B.
Ohio State University
Department of Forestry
Ohio Agricultural Research
 and Development Center
Wooster, OH 44691
(216) 263-3783

Krutovskii, Konstantin
N. I. Vavilov
Institute of General Genetics
USSR Academy of Sciences
Gubkin St. 3, 117809 GSP-1
Moscow B-333
RUSSIA
(7) 135-50-67

Kuhlman, E. George
USDA Forest Service
Southeastern Forest
  Experiment Station
Forestry Sciences Laboratory
Green/Carlton Street
Athens, GA 30602
(404) 546-2455

Kung, Fan H.
Dept. of Forestry
Southern Illinois University
Carbondale, IL 62901
(618) 453-7460

LaFarge, Timothy
USDA Forest Service
1720 Peachtree Road, NW
Atlanta, GA 30367
(404) 347-4046

Lambeth, Clem
Smarfit Group
P.O. Box 626
Callahan, FL 32011
(904) 879-3051

Land, Samuel B., Jr.
Mississippi State University
P.O. Drawer FR
Mississippi State, MS 39762
(601) 325-2946

Lantz, Clark W.
USDA Forest Service
1720 Peachtree Road, NW
Atlanta, GA 30367
(404) 347-3554

Leach, Gregory N.
Champion International Corp.
P.O. Box 875
Cantonment, FL 32533
(904) 968-3050

Lee, Mike
Mississippi Forestry Commission
P.O. Box 468
Lumberton, MS
(601) 796-8892

Li, Bailian
University of Minnesota
NC Experiment Station
1861 Hwy 169, East
Grand Rapids, MN 55744
(218) 327-4490

Lowe, W. J.
Texas Forest Service
Forest Science Lab
College Station, TX 77843
(409) 845-2523

Mahalovich, Mary F.
USDA Forest Service
310 W. Wisconsin Avenue
Milwaukee, WI 53203-2291
(414) 297-3693

Manga, Vinod Kumar
Mississippi State University
P.O. Drawer FR
Mississippi State, MS 39762
(601) 325-2946

Marland, Gregg
Environmental Sciences Division
Oak Ridge National Laboratory
P.O. Box 2008
Oak Ridge, TN 37831-6335
(615) 574-0390

Martin, Jeanne A.
California Department of Forestry
P.O. Box 1590
Davis, CA 95617
(916) 753-2441

Massie, William E., Jr.
Mississippi Forestry Commission
P.O. Box 468
Lumberton, MS 39455
(601) 796-8892

Mathis, James N.
Institute of Paper
  Science and Technology
575 14th Street, NW
Atlanta, GA 30318
(404) 853-9787

Maynor, Maxie
Federal Paper Board
P.O. Box 1007
Lumberton, NC 28359
(919) 739-7596

McCall, Early
ITT Rayonier Inc.
Forest Products
P.O. Box 819
Yulee, FL 32034
(904) 225-5393

McConnell, James   L.
USDA Forest Service
1720 Peachtree Road, NW
Atlanta, GA 30367
(404) 347-4045

McCutchan, Barbara G.
Westvaco Corporation
P.O. Box 1950
Summerville, SC 29484
(803) 871-5000

McKeand, Steve
North Carolina State University
Box 8002
Raleigh, NC 27695-8002
(919) 737-3168

McKinley, Craig R.
Texas Forest Service
Forest Genetics Lab - TAMU
College Station, TX 77843
(409) 845-1296

McMahen, Paul
Willamette Industries, Inc.
General Delivery
Taylor, LA 71080
(318) 377-4465

McRae, John B.
International Forest Seed Company
P.O. Box 490
Odenville, AL 35120
(205) 629-6461

Merkle, Scott A.
University of Georgia
School of Forest Resources
Athens, GA 30602
(404) 542-6112

Miller, Larry G.
Temple-Inland Forest Products Corp.
229 N Bowie
Jasper, TX 75951
(409) 384-3434

Morrow, Daniel F.
Boise Cascade Corporation
P.O. Box 37
Singer, LA 70660
(318) 463-9681

Muona, Outi
Department of Genetics
University of Oulu
90570 Oulu
FINLAND
358-81-353303

Murray, Gordon
Forestry Canada
Petawawa National Forestry Institute
P.O. Box 2000
Chalk River, Ontatio
CANADA K0J 1J0
(613) 589-2880

Nance, Warren L.
USDA Forest Service
Southern Forest Experiment Station
Box 2008, GMF
Gulfport, MS 39503
(601) 864-3972

Nelson, C. Dana
USDA Forest Service
Southern Forest Experiment Station
Box 2008, GMF
Gulfport, MS 39503
(601) 864-3972

Oppenheimer, Gary
Weyerhauser Company
Route 3, Box 365
Washington, NC 27889

Patel, Rupal
University of Kentucky
Department of Forestry
Lexington, KY 40546-0073
(606) 223-4597

Pitcher, John A.
Hardwood Research Council
P.O. Box 34518
Memphis, TN 38184-0518
(901) 377-1824

Pohl, Russell
Georgia Forestry Commission
1843 Kirkwood Drive
Macon, GA 31211
(912) 744-3383

Porterfield, James David II
Oklahoma Forestry Division
Route 1, Box 44
Washington, OK 73093
(405) 288-2385

Powell, Gregory L.
University of Florida
118 Newins-Zieger
Gainesville, FL 32611
(904) 392-1850

Robison, Terry L.
Missouri Department of Conservation
2901 W. Truman Blvd.
Jefferson City, MO 65109
(314) 751-4115

Rousseau, Randy T.
Westvaco Corporation
P.O. Box 458
Wickliffe, KY 42087
(502) 335-3151

Rudie, Alan W.
Institute of Paper Science
  and Technology
575 14th Street, NW
Atlanta, GA 30318
(404) 853-9706

Salazar, Mauro
North Carolina State University
E.S. King Village M-13
Raleigh, NC 27607
(919) 821-1109

Schlarbaum, Scott E.
University of Tennessee
Department of Forestry,
  Wildlife & Fisheries
Knoxville, TN 37901-1071
(615) 974-7126

Schmidtling, Ron
USDA Forest Service
Southern Forest
  Experiment Station
P.O. Box 2008 GMF
Gulfport, MS 39505
(601) 864-3972

Schumann, Carol
USDA Forest Service
Forestry Sciences Laboratory
University of Nebraska - East Campus
Lincoln, NE 68583-0822
(402) 437-5178

Schwarz, Otto J.
University of Tennessee-Knoxville
Department of Botany
Knoxville, TN 37991-1100
(615) 974-2256

Sharma, Narinder Kumar
Mississippi State University
P.O. Drawer FR
Mississippi State, MS 39762
(601) 325-2946

Singh, Gyanendra
Mississippi State University
P.O. Drawer FR
Mississippi State, MS 39762
(601) 325-2946

Slichter, Timothy K.
International Paper Co.
4189 Bellamy Bridge Road
Marianna, FL 32446
(904) 594-6001

Sluder, Earl R.
USDA Forest Service
Southeastern Forest
  Experiment Station
Route 1, Box 182A
Dry Branch, GA 31020-9801
(912) 744-0261

Smith, C. Ken
University of Florida
2490 SW 14th Drive #18
Gainesville, FL 32608
(904) 335-9060

Sommer, Harry E.
University of Georgia
School of Forest Resources
Athens, GA 30602
(404) 542-2535

Steele, James A.
Temple-Inland Forest
  Products Corporation
229 N. Bowie
Jasper, TX 75951
(409) 384-3434

Stelzer, Hank
Procter & Gamble Cellulose
Route 3, Box 260
Perry, FL   32347
(904) 584-0326

Stine, Michael
Louisiana State University
School of Forestry,
   Wildlife & Fisheries
Baton Rouge, LA   70803
(504)   388-4137

Stomp, Anne M.
North Carolina State University
Forestry Dept., U-Box 8002
Raleigh, NC   27695-8002
(919) 737-7572

Studyvin, Charles
USDA Forest Service
Box 255
Mt. Ida, AR   71957
(501)   867-2101

Sung, Shi-Jean (Susana)
USDA Forestry Service
Southeastern Forest
   Experiment Station
Forestry Sciences Laboratory
Carlton/Green Streets
Athens, GA   30602
(404)   546-2435

Surles, Scott
University of Florida
Department of Forestry
118 Newins-Ziegler Hall
Gainesville, FL   32811
(904) 392-1850

Tang, Zhenmin
Mississippi State University
P.O. Drawer FR
Mississippi State, MS   39762
(601)   325-2946

Taylor, Robin W.
USDA Forest Service
201 Woodland Drive
Murphy, NC   28906
(704)   837-5152

Thor, Eyvind
University of Tennessee
Department of Forestry,
   Wildlife & Fisheries
Knoxville, TN   37901-1071
(615) 974-7126

Thornburg, Robert W.
Iowa State University
Department of Biochemistry
   and Biophysics
Ames, Iowa   50011
(515)   294-0907

Tianquan, Li
University of Kentucky
Department of Forestry
Lexington, KY   40546-0073
(606)   257-7657

Tibbs, Tom
USDA Forest Service
1720 Peachtree Road, NW
Atlanta, GA   30367
(404)   347-3946

Todd, S. David
Champion International
   Corporation
37 Villa Road,
   Suite 319, B-141
Greenville, SC   29615
(803)   370-7214

Tolentino, Enrique L., Jr.
Mississippi State University
P.O. Drawer FR
Mississippi State, MS   39762
(601)   325-2946

Tule, James O.
International Paper Company
P.O. Box 2099
Woodville, TX   75979
(409)   283-7493

Tuskan, Gerald A.
Oak Ridge National Laboratory
P.O. Box 2008, Bldg 1503
Oak Ridge, TN   37831
(615)   576-8141

van Buijtenen, J. P.
Texas Forest Service
Texas A&M University
College Station, TX   77843-2135

Vasquez, Jorge
North Carolina State University
P.O. Box 33503
Raleigh, NC 27636
(919) 832-5247

Vermillion, Tom
Cavanham Forest Industries Inc.
Route 3, Box 247A
Bogalusa, LA 70427
(504) 732-6750

Wagner, David B.
University of Kentucky
Dept. of Forestry
204 T.P. Cooper Bldg.
Lexington, KY 40546-0073
(606) 257-7596

Wann, Steven R.
Union Camp Corporation
P.O. Box 3301
Princeton, NJ 08543-3301
609-896-1200

Weir, Robert J.
North Carolina State University
Box 8002
Raleigh, NC 27695-8002
(919) 737-3168

Welch, Richard V.
309 Chappel Hill Road #5
Hot Springs, AR 71913-6699
(501) 525-1598

White, Gordon
5020 Grizzard Road
Huntsville, AL 35810
(205) 852-4911

White, Timothy L.
University of Florida
118 Newins-Ziegler Hall
Gainesville, FL 32611
(904) 392-1850

Wilcox, Phillip L.
North Carolina State University
Box 8008
Raleigh, NC 27695-8008
(919) 737-7800

Wilde, Dayton
School of Forest Resources
University of Georgia
Athens, GA 30602
(404) 542-6515

Williams, Claire G.
Weyerhauser Company
New Bern R & E Field Station
P.O. Box 1391
New Bern, NC 28560
(919) 633-7212

Williford, Mike
Bowater Inc., Southern
  Division Woodlands
Route 4, Box 41519
Chatsworth, GA 30705
(404) 334-2422

Windham, Jerry W.
USDA Forest Service
368 Ashe Nursery Road
Brooklyn, MS 39425
(601) 584-8488

Wise, Farrell
Westvaco Corportion
P.O. Box 1950
Summerville, SC 29484
(803) 871-5000

Wiselogel, Arthur
Solar Energy Research Institute
Chemical Conversion Division
1617 Cole Blvd
Golden, CO 80401

Wood, Michael
Institute of Paper Science
  and Technology
575 14th Street
Atlanta, GA 30318
(404) 853-9785

Zamudio, Francisco
North Carolina State University
  at Raleigh
CAMCORE
P. O. Box 7626
Raleigh, NC 27695-7626
(909) 737-2739

Zoerb, Marvin
Union Camp Corporation
Box 216
Rincon, GA   31326
(912)   826-5556

SOUTHERN FOREST TREE

IMPROVEMENT COMMITTEE MEMBERS

SOUTHERN FOREST TREE IMPROVEMENT COMMITTEE

Membership List (Effective for 1990 annual meeting)

June 1990

GROUP A
(Representatives Appointed for 6-Year Term)

Committee Membership	Term Ends (At end of Annual Meeting)	Representing
Jack A. Pitcher Hardwood Research Council P.O. Box 34518 Memphis, Tennessee 38184-0518 (901) 377-1824	1995	Hardwood Research Council
Randy Rousseau Forest Geneticist Westvaco Corp. P.O. Box 458 Wickliffe, Kentucky 42087 (502) 335-3156	1995	Forest Industry
Siroos T. Jahromi International Paper Company Southlands Experiment Forest Route 1, Box 571 Bainbridge, Georgia 31717 (912) 246-3642	1992	Forest Industry
Mike Waxler Southern Operations Geneticists Weyerhauser Corporation Box 1060 Hot Springs, Arkansas 71902 (501) 624-8484	1992	Forest Industry

Committee Membership	Term Ends (At end of Annual Meeting)	Representing
Garner Barnum Management Forester Arkansas Forestry Commission P.O. Box 4523, Asher Station 3821 W. Roosevelt Road Little Rock, Arkansas 72214 (501) 664-2531	1994	State Forestry Agencies
Russell Pohl Georgia Forestry Commission Box 819 Macon, Georgia 31298-4599 (912) 744-3354	1992	State Forestry Agencies
Bill Padgett Alabama Forestry Commission 513 Madison Avenue Montgomery, Alabama 36130-0601 (205) 240-9304	1994	State Forestry Agencies
Dr. Peter Feret Virginia Polytechnic Institute Department of Forestry 228 Cheatham Hall Blacksburg, VA 24061 (703) 961-5943	1995	Forestry Schools
Dr. Sam Land Mississippi State University School of Forest Resources Department of Forestry P.O. Drawer FD State College, MS 39762 (601) 325-2946	1992	Forestry Schools

David B. Wagner   1994   Forestry Schools
Dept. of Forestry
University of Kentucky
Lexington, Kentucky 40546
(606) 257-3773

James Hodges   1992   Forest Industry
Champion International Corp.
Eastern Seed Orchard
P.O. Box 97
Tillery, North Carolina 27887
(919) 826-4182

GROUP B

(Representatives Appointed for Indefinite Terms)

<u>Committee Membership</u>   <u>Representing</u>

Warren Nance   USFS-Southern Forest
Institute of Forest Genetics   Experiment Station
P.O. Box 2008, GMF
Gulfport, Mississippi 39503
(601) 864-3972

Clark Lantz   USFS-State & Private
Cooperative Forestry   Forestry
U. S. Forest Service
Suite 811
1720 Peachtree Road, N. W.
Atlanta, Georgia 30367
(404) 881-3551

Floyd Bridgwater   USFS-Southeastern
USDA Forest Service   Forest Expt. Station
Southeastern Forest
  Experiment Station
College of Forest Resources
North Carolina State University
Box 8002
Raleigh, North Carolina 27695-8002

GROUP C

(Specialists Appointed for 6-Year Term)

Committee Membership	Term Ends (At end of Annual Meeting)	Representing
Bob Schmidt, IPM School of Forest Resources University of Florida Gainesville, Florida 32611 (904) 392-4826	1993	Pathology
Harry O. Yate Southeastern For. Expt. Sta. Carlton Street Athens, Georgia 30601 (404) 546-2467	1990	Entomology
Dave Bramlett U. S. Forest Service Route 1, Box 182A Dry Branch, Georgia 31020 (912) 744-0261	1993	Pollen Management
Warren Nance Institute of Forest Genetics Box 2008, GMF Gulfport, MS 39503 (601) 864-3972	1990	Stand Dynamics
Ron Schmidtling Institute of Forest Genetics Box 2008, GMF Gulfport, MS 39503 (601) 864-3972	1992	Racial Variation and Seed Movement
J. B. Jett Forestry Department Box 8002 North Carolina State University Raleigh, NC 27695-8002 (919) 737-3168	(When regional seed certifi-cation plan completed)	Seed Certification

Bill Lowe
Associate Geneticist
Western Gulf Forest Tree
   Improvement Program
Forest Science Laboratory
College Station, Texas 77843-2585
(409) 845-2556

(When subcommittee
work complete)

Seed Orchard Pest
Management Sub-
committee